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4205

Nitrogen Metabolism of Diabetic Children.

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(Introduced by J. D. Boyd.)

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The nitrogen retention of a group of 33 diabetic children between the ages of 3 and 15 years has been studied during a total of 42 metabolism periods of 3 to 4 days each. The diets were calorically sufficient according to Holt's standards, and the protein intake approximated 2 gm. per kilogram body weight, the average for the younger children being slightly above, and that of the older group somewhat below this value.

The average retention of 4 children more than 10% under weight was greater than that of children whose weight was approximately normal, while the retention of overweight children did not differ materially from that of the normal group. The average retention for the group (omitting the 4 underweight children) was .044 gm. of nitrogen per kilogram body weight.

4206

Endogenous Rickets.

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Active rickets has been observed in a number of patients whose intake of vitamins, calcium and phosphorus was fully adequate,

according to our present knowledge of such requirements. Prolonged use of potent cod liver oil failed to induce healing. In each case, the blood serum calcium values were approximately normal, the phosphorus being markedly lowered. In this respect they differ from the findings with the rickets which may be associated with renal inadequacy, in which the calcium is low, the phosphorus normal or increased. Because of absence of any demonstrable exogenous etiology, these cases have been considered as of endogenous origin. While rachitic symptoms were sufficiently marked in each patient to demand medical attention, laboratory studies indicated the presence of other metabolic disturbances in each case. Among the diverse syndromes presented by these patients are included atypical diabetes mellitus, diabetes insipidus, and extrophy of the bladder with transplantation of the ureters to the rectum. Patients studied intensively showed disturbances of the acid-base balance, of varying type and degree. In one instance the rickets became inactive after sodium bicarbonate, 1 gm. 3 times daily, was added to the patient's previously ineffective antirachitic regime. The data indicate that probably an actual or relative base deficit was present in each case.

4207

Effect of Intravenous Injections of Alkali on Physiological Action of Curare.

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In an earlier paper[1] it was shown that a decrease in the pH of the blood, produced prior to the administration of lethal doses of strychnine, prevented violent tetanus and death. It was considered important to try the reciprocal experiment, that is, the effect of administering alkali to dogs that had previously received a lethal dose of curare.

Dogs used in this experiment received, intravenously, 5.7 mg. of curare per kilo of body weight. The drug used was the product of Burroughs, Wellcome & Co. Eight or 10 minutes after injection, when paralysis of the muscles, except the respiratory muscles, had appeared, a 5% solution of $NaHCO_3$ was injected intravenously. Complete recovery occurred within 15 minutes after approximately

[1] Wenner, W. F., and Blanchard, E. W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 726.

3.0 gm. of $NaHCO_3$ had been introduced. The "curare effect" did not reappear.

At the onset of muscle paralysis the pH and CO_2 capacity begin to fall. The acidosis produced evidently augments the effect of curare but is not, in itself, sufficiently pronounced to produce coma. Introduction of $NaHCO_3$ not only corrects anoxemia but also possibly neutralizes the curare effect at the myo-neural junction.

All controls died of respiratory paralysis within 20 minutes after receiving curare.

The following table gives the data from one of our experimental animals:

TABLE I. Dog No. 5, weight 7.5 kg.

Time	Amt. Curare given	Amt. $NaHCO_3$ given	Blood pH	CO_2 Capacity Vol. %	Remarks.
10:40			7.39	42.0	
10:42	40.5 mg.				Injected intravenously.
10:50			7.27	40.2	Muscle paralysis. Dog still breathing.
10:51 to 10:53		2.5 gm.			Injected intravenously.
10:55					Reflexes returned.
11:15			7.46	47.6	Complete recovery.

4208

Histolytic Influence of Atrophying Gills During Metamorphosis: Special Reference to Resistance of Fore-limb Integument.

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From the Zoological Laboratory, State University of Iowa.

Helff[1] has shown that the perforations in the opercular integument are the end result of an orderly process of cellular histolysis, due to the histolytic action of the atrophying gills. The present investigation was designed to determine the stage of gill atrophy at which this histolytic influence is most effective.

Homoplastic transplants of gills in various stages of atrophy were made to regions beneath the back skin of metamorphosing *Rana pipiens* tadpoles. The following constitute the main stages

[1] Helff, O. M., *J. Exp. Zool.*, 1926, xlv, 1.

of gill atrophy investigated: (1) a stage showing the earliest macroscopic signs of atrophy; (2) a stage just prior to the release of the fore-limb; (3) a much later stage in which the integuments of the opercular region and the fore-limb had fused, following the release of the latter. Other atrophying gill transplants were made of stages intermediary to these, but the differences in the histolytic action were more clear cut when comparing the transplantation results of the 3 stages mentioned above.

Typical perforations were formed in the skin of the back directly in contact with the gill transplants. Histological sections of integument bounding such perforations showed that histolysis had taken place, identical to normal opercular integumentary histolysis. The stage of greatest histolytic influence of the atrophying gill was determined by the length of time necessary for the transplant to form a uniform sized perforation in the integument. This stage was found to be that just prior to the breaking through of the fore-limb. At this stage the gills are of a reddish hue, quite mushy in consistency, and capable of producing a perforation within 2 days.

The results indicate that the histolytic influence of the atrophying gills is cumulative. It is very weak during the early stages of gill atrophy, but increases proportionately with increasing atrophy and reaches a maximum just prior to the release of the fore-limbs. Following the release of the fore-limbs the histolytic influence of the gills still persists, but gradually subsides as the gills undergo their final stages of atrophy.

During metamorphosis the fore-limbs differentiate very rapidly and occupy a large part of the opercular cavity. They were in contact with the atrophying gills which appear, however, to be ineffective in producing histolysis of the fore-limb integument. Operations were designed to throw light on this apparent resistance of fore-limb integument to histolysis.

In determining the possible specificity of fore-limb integument in this respect, autoplastic fore-limb integument transplantations were made to regions of the back. Homoplastic gill transplants, in the stage of greatest histolytic influence were then placed beneath the skin grafts. In this manner atrophying gill tissue was brought in contact with the *inner surface* of the fore-limb integument, a condition never attained during the normal development of fore-limb integument in the opercular cavity.

Perforations appeared in the skin grafts in all cases, being preceded by a definite process of thinning corresponding closely to the macroscopic thinning of normal opercular integument. The thinning was first noticeable in the integument directly over the slight

bulge caused by the gill transplantation, and resulted in a loss of coloration, the area assuming a grayish and opalescent shade typical of histolysis. Histological sections later showed that the perforations were the result of a definite and typical cellular histolysis.

Homoplastic gill transplants in stages of greatest histolytic activity were next placed on the epidermal surface of fore-limb integument grafts, which had been previously transplanted to the back. Normal back skin was then grafted over both the gill and fore-limb integument grafts. After a typical perforation had appeared in the back skin graft, the 2 skin grafts were removed and sectioned. It was at once evident that the fore-limb integument showed only slight sign of histolysis as compared with the total disintegration of the back skin where the perforation had formed.

The above results indicate strongly that the failure of the integument of the fore-limbs to histolyze during metamorphosis when in contact with the atrophying gills, is due to the fact that the gills are in contact with the *epidermal surface* of the skin only. It must also be borne in mind that the integument of the fore-limb is undergoing rapid growth and development during metamorphosis and that the heightened metabolism of this tissue would also probably tend to counteract the histolyzing influence of the atrophying gills.

4209

Influence of Wheat Germ Oil on Fertility of Rats Fed Fat Free Rations.

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In animal studies pertaining to Vitamin E, varying amounts of fat have been used as essential constituents of the ration. Certain of these are known to have a specific influence on the rate of the oxidative changes in fats carrying the vitamins A and E. For example, wheat germ oil, a substance seemingly rich in Vitamin E, has been found to retard the oxidation of cod liver oil, as well as the destruction of Vitamins A and E; whereas lard hastens the destruction of these vitamins.[1] Correlating these findings with the

[1] Mattill, H. A., *J. Am. Med. Assn.*, 1927, lxxxix, 1505. Evans, H. M., and Burr, G. O., *J. Am. Med. Assn.*, 1927, lxxxviii, 1462. Fredreica, L. S., *J. Biol. Chem.*, 1924, lxii, 471.

results of animal experiments,[2] one is led to wonder if wheat germ oil is effective as an antisterility agent because of the presence of a specific vitamin, or because of its retarding influence on the oxidation of Vitamin A, a certain minimum of which is necessary for reproduction.

It seemed possible that this might be determined by comparing the growth and reproductive behavior of animals receiving fat free rations, with animals receiving similar rations to which were added small amounts of wheat germ oil. We therefore have fed 3 groups of rats purified rations in which the Vitamins A and D were supplied by a cod liver oil concentrate (Oscodol*). Vitamin B was furnished by starch free yeast.[3] In one group the ration was supplemented with 3% of wheat oil[4]; in another 12 gm. of lard was substituted for 27 gm. of cornstarch. All rations were made into a paste with distilled water, fresh mixtures being prepared approximately twice a week. Two oscodol tablets, the equivalent of about 5% of the ration in cod liver oil, were added to every 488 calories of food. This, we have reason to believe, furnished a plethora of Vitamin A.[5] Each experimental group, consisting of 2 males and 4 females, was placed on the ration when the animals were about 4 weeks of age.

The growth curves of all the animals were comparable, and were somewhat better than the Donaldson curves for normal rats. There were no symptoms of Xerophthalmia or upper respiratory infection. The data pertaining to the composition of the ration, and the results of the feeding tests are given in the following table.

Only one female in the group receiving the ration containing no fat (Group A) reproduced. This animal which weighed 60 gm. at the beginning of the experiment was about 6 days older than the others. The physiological changes resulting from deprivation of Vitamin E had not occurred at this time. There was no second gestation. In contrast to Group A is Group B, which received a similar ration, but with added wheat germ oil. The reproductive performance of these rats is fairly comparable to that of our stock animals. Within 7 months each female produced 2 litters; one pro-

[2] Nelson, V. E., Jones, R. L., Heller, V. G., Parks, T. B., and Fulmer, E. I., *Am. J. Physiol.*, 1926, lxxvi, 325. Nelson, V. E., Heller, V. G., and Fulmer, E. I., *J. Biol. Chem.*, 1923, lvii, 415. Anderegg, L. T., and Nelson, V. E., *J. Indust. and Engin. Chem.*, 1925, xvii, 451.

* Supplied through the courtesy of H. A. Metz Laboratories.

[3] Furnished by Fleishman Yeast Company.

[4] An extract of wheat germ furnished by Squibb and Company.

[5] Steenbock, H., Jones, J. H., and Hart, E. B., *J. Biol. Chem.*, 1923, lviii, 383. Dubin, H. E., and Funk, C., *J. Metab. Res.*, 1923, iv, 467.

TABLE I. *Influence of Wheat Germ Oil on Fertility.*

Group	Ration	No. of females	Initial wt. av. gm.	Av. No. of pregnancies to 7 mos.	Av. age at birth of young mother	Av. No. of young per female	Viable young %	Remarks
A	Fat free*	4	47.7	0.25	4	1.7	86	Rat which reproduced weighed 60 gm. at beginning of experiment.
B	Fat free + 3% wheat germ oil	4	41.	2.25	3	15.	78	One mother died; cause unknown.
C	Fat free + lard†	4	45.5	1.	3	3.5	7.1‡	Two litters died at birth; another within 24 hours; one mother died.

*The ration consisted of 22 gm. of casein, 100 gm. of cornstarch, 7 gm. of yeast, 8.5 gm. of a salt mixture, 1.5 gm. or iron citrate, and 0.5 cc. of a 2% potassium iodide solution. 2 oscodol tablets were crushed, mixed with water, and added to the mixture.

†12 gm. of lard were substituted for 27 gm. of cornstarch.

‡When 3 weeks old this animal developed convulsions. It was given wheat germ oil by mouth several times a day for a period of one week.

duced 3. The average number of young per litter was 6.4. With the lard and oscodol (Group C) the results were slightly better than with the fat free ration, but in no way comparable to those receiving the wheat germ oil. Each female reproduced once. Two litters

died at birth or were born dead. These were not seen. A third litter died within 24 hours after birth. Reproduction in these would seem to be an example of delayed sterility, for which we have no explanation. Evans and Burr[6] cite similar cases. In this group also there were no second gestations.

The results of the investigation quite definitely indicate that the effectiveness of wheat germ oil as an anti-sterility agent is due to the presence of some substance (Vitamin E) contained therein, and not to any retarding action which this may have on the oxidation of Vitamin A.

4210

Effect of Changes in Ion Concentration of Blood upon Reflex Time.

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Previous studies[1, 2] on the effect of changes in acid-base equilibrium on the physiological action of strychnine and curare indicated that an increase in hydrogen or hydroxyl ions might affect neural or neuro-muscular factors and thus alter the rate of conduction of the nerve impulse.

Dogs were used as experimental animals. The patellar tendon reflex time of normal unanesthetized dogs varied from .00775 to .0094 seconds with an average of .0082 seconds. The pH of the blood of the normal dogs at the time the records were obtained was about 7.36 and the CO_2 capacity 45 volumes %. With a drop in pH and CO_2 capacity the reflex time was decreased to about .0043 seconds. An increase in pH and CO_2 capacity also brought about a decrease in reflex time. The average reflex time following alkali administration was .0057 seconds. One dog included here showed an insignificant increase of .0006 seconds in reflex conduction rate at pH 7.53 and CO_2 capacity 72.97 volumes %.

The records obtained show clearly that the reflex time of dogs is markedly reduced, following acid, and to a less degree, following alkali administration.

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[6] Evans, H. M., and Burr, G. O., loc. cit.

[1] Wenner, W. F., and Blanchard, E. W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 726.

[2] Wenner, W. F., and Blanchard, E. W., *Ibid.*, 1928, xxvi, (preceding abstract).

It is suggested that an increase in H ions probably blocks out the higher centers ordinarily involved in the arc. On the other hand OH ions probably aid in facilitating the conduction of the nerve impulse.

4211

Aspects of Mineral Nutrient Balance as Related to Sap Hydrion Concentration.

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From the Department of Botany, State University of Iowa.

Fluctuations in amounts of mineral nutrients found in plants is known to exert a profound effect on their metabolism. Results of recent experiments with grain plants grown on a number of acidic, humus soils deficient in potash disclose striking effects of certain mineral nutrients.

The accompanying chart (Fig. 1), typical of conditions in a number of grains, depicts schematically the chemical analyses of 3 oat crops *(Avena sativa)* grown on a soil variously treated with mineral

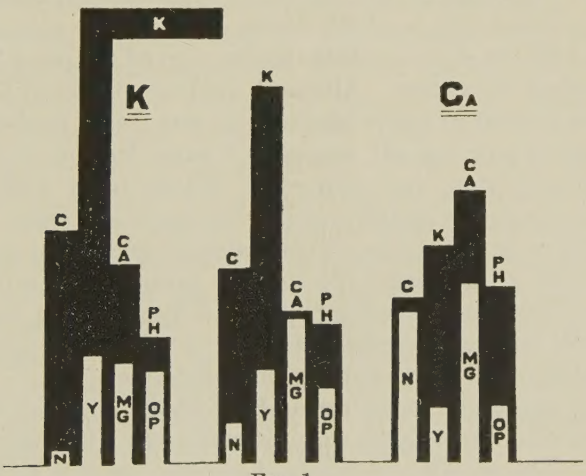

FIG. 1.

Analyses of entire oat plants grown in a potash deficient, acid humus following treatment with nutrients as follows: Left group (K), 400 p.p.m. of powdered KCl; center group, untreated (check) soil; right group (Ca), 4000 p.p.m. $CaCO_3$. c, total hydrolyzable carbohydrates; n, nitrate nitrogen; k, potassium; y, yield as dry weight; ca, calcium; mg, magnesium; all given as percentage of plant dry weight. ph, sap acidity in pH units; OP, osmotic pressure of expressed sap in atmospheres.

fertilizers. Variation in the amount of potassium is readily apparent. Correlated with decreases in potash is a fall, often greater than 3 pH units, in a sap hydrion concentration, a variation of sufficient magnitude to distinctly alter the solubility and, hence, also the mobility of essential nutrients in plants. The effects of impaired translocation are thus apt to be superimposed upon those of potassium insufficiency. External symptoms of injury and the results of tissue analyses support this view.

Potash starvation is known to interfere with carbohydrate storage, a process of fundamental significance in plants. Concomitant with decreases in potassium there was observed a decrease in hydrolyzable carbohydrates and an increase in nitrates. The great increase in soluble nitrates during potash starvation suggests a carbohydrate insufficiency severe enough to impede protein synthesis. This is true even though allowance is made for nitrates which accumulate in soils in the presence of lime. Consideration of this phenomenon is especially pertinent because lime was abundant in soils and tissues low in potash. In fact, the data (Fig. 1) show that potassium on the one hand is rather delicately balanced by calcium and magnesium on the other, an increase in the former entailing a diminution in the latter and vice versa.

Effects of potash insufficiency were accentuated by depressed iron mobility in plants whose sap had a low hydrogen-ion concentration. Inability to translocate insoluble forms of iron from roots to foliage interferes with the photosynthetic mechanism of the plant by impairing chlorophyll formation. Although analyses disclosed little variation in iron content of entire plants, iron was found massed in roots of specimens whose sap pH was near 7 while foliage of these same plants suffered from iron chlorosis. Plants from acid soils had their iron more evenly distributed between roots and tops.

Diminutions in potassium content were coupled with a fall in osmotic pressure of extracted cell sap due primarily to a decrease in the total soluble mineral content. Thus it appears that the mobility of inorganic solutes in general as well as of iron in particular is diminished by a fall in sap hydrion concentration.

Extreme variations in the ratio of the above nutrients to one another were often associated with characteristic external symptoms of injury. The severity of injury, however, appears to be a function not only of the balance among essential nutrients but also of their absolute amounts. Only when some constituent fell below a critical minimum did injury become apparent in plants. A falling off in dry weight yield due to lack of nutrient balance usually precedes the appearance of other symptoms of injury.

4212

Metamorphosis of the Colorado Axolotl by Injection of Inorganic Iodine.

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Experiments with the Colorado Axolotl, to be properly controlled, must be performed with hypophysectomized and thyroidectomized individuals, since this variety of axolotl metamorphoses spontaneously when removed from its native habitat. Individuals submitted to such treatment have been considered never to metamorphose except under the influence of a glandular replacement therapy or upon the administration of organic iodine compounds. Jensen,[1] Huxley and Hogben,[2] Huxley,[3] and Uhlenhuth,[4] in view of their experiments maintain that the transformation of urodeles cannot be accelerated by feeding inorganic iodine. However, Hirschler[5] was successful in bringing about the metamorphosis of one European Axolotl by injection of Lugol's solution. This type of axolotl never metamorphoses spontaneously. Blacher and Belkin[6] reported normal and hypophysectomized European axolotls brought to the adult condition by intraperitoneal implantations of small crystals of inorganic iodine.

The writer has conducted somewhat similar experiments with the Colorado Axolotl, and, following the subcutaneous implantation of powdered iodine crystals, hypophysectomized and thyroidectomized individuals rapidly passed through the transformation stages to the adult. The experimental animals were either kept long enough after operation to preclude the possibility of small bits of glandular tissue remaining, or were killed following metamorphosis and the gland sites carefully examined. The controls all metamorphosed spontaneously within a month after arrival at the laboratory.

These results confirm the observations of Blacher and Belkin upon the European Axolotl. Although most investigators have considered inorganic iodine to have negligible influence upon the metamorphosis of urodeles, it now appears that large amounts of this element administered subcutaneously or intraperitoneally do markedly accelerate differentiation in neotenic forms.

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1 Jensen, C. O., *C. R. Soc. Biol.*, 1921, lxxv, 391.

2 Huxley, J. S., and Hogben, L., *Proc. Roy. Soc.*, B. 1922, xciii, 36.

3 Huxley, J. S., *Proc. Roy. Soc.*, B. 1925, xcviii, 113.

4 Uhlenhuth, E., *Biol. Bull.*, 1922, xlii, 143; *ibid.*, 1923, xliv-xlv, 303.

5 Hirschler, J., *Arch. Entw. Organ.*, 1922, 51, 482.

6 Blacher, L. J., and Belkin, R. I., *Trans. Lab. Exp. Biol, of Zoopark of Moscow*, 1927, iii, 97.

New York Meeting.

College of Physicians and Surgeons, December 19, 1928.

4213

Note on the Penetration of Electrolytes.

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When a weak electrolyte penetrates a living cell the distribution at equilibrium[1] between the inside and outside does not tell us whether it enters the cell in ionic or molecular form, for the equilibrium is the same in both cases, being determined by the fact that the ionic activity product (or chemical potential) must be the same inside and outside. Assuming that the dissociation constants are the same inside and outside and that the activity coefficients equal 1 we may write for a weak acid, HA,

$$\frac{(H_i)\ (A_i)}{M_i} = K = \frac{(H_o)\ (A_o)}{M_o}$$

where the subscripts o and i denote the external and internal concentrations respectively, and M denotes undissociated molecules. Since the ionic product inside must be equal to that outside we have $(H_i)\ (A_i) = (H_o)\ (A_o)$ and therefore $M_i = M_o$.[2]

For convenience we may consider a cylindrical plant cell in contact with a solution at one of its end surfaces, the content of its central vacuole being well stirred (which may sometimes happen as

[1] This was mentioned in a lecture before the Mayo Clinic in 1927 (*cf.* Osterhout, W. J. V., Some aspects of cellular physiology, in Mayo Lectures, 1928) and differs from the view expressed in earlier papers.

When there is no Donnan equilibrium there is no true equilibrium as long as there is a difference between the pH value of the sap and the external solution, but a weak acid may very quickly come to an apparent equilibrium inside and outside as has been found experimentally (*e. g.*, in the case of CO_2 and H_2S, *cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1925-28, viii, 131; Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925-26, ix, 255).

[2] For the purposes of this paper we need not consider the internal concentration of ions but it can always be found if we know the pH value of the sap, M_i and K.

the result of protoplasmic motion) : we may assume that the external solution is likewise stirred and has a large volume so that it remains practically constant during penetration. For convenience we shall assume that the pH value is the same inside and outside and remains practically constant during the experiment.

If molecules alone enter and the quantity passing in be called x it is evident that the observed rate $\frac{dx}{dt}$ will be proportional to the net rate of passage of molecules through the protoplasm,[3] D_M, to the permeability of the protoplasm to molecules, P_M, and to the difference between the external concentration of molecules, M_o, and the internal concentration found in the vacuole, M_i. Hence we may write $\frac{dx}{dt} = D_M P_M (M_o - M_i)$. This is similar to the formula employed by Northrop for diffusion through collodion membranes in contact with solutions.[4] Putting $D_M P_M = V_M$ (the apparent velocity constant of the process) we have[5]

Let us now consider what will happen if ions alone enter. Unless there is an exchange of ions going in opposite directions[6] H^+ cannot enter without A^- so that we may speak of the penetration of ion pairs ($H^+ + A^-$). In order that an ion pair may enter H^+ and A^-

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[3] The cell wall will be left out of consideration in the present discussion.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1928-29, xii, 435. The formula employed by Northrop is $Q = \frac{t\ D\ AS\ (C_1 - C_2)}{h}$, where Q is the quantity passing in in the time t, D is the diffusion constant in the collodion, S is the partition coefficient between collodion and water, A is the area, and h the thickness of the collodion membrane and C_1 and C_2 are the external and internal concentrations of molecules. The S of Northrop's formula might be regarded as analogous to the P in our formula but evidently P cannot be regarded simply as a partition coefficient since the protoplasm is not a single phase; we regard P as a proportionality factor representing the number of molecules passing through the protoplasm as compared with the number striking the surface (*e. g.*, if half the molecules striking the surface enter, P will be equal to 0.5). We might regard the permeability of the protoplasm as depending also on D_M but it seems simpler not to do this. D_M may be regarded as the reciprocal of the time required to pass through the protoplasm and hence takes account of its thickness as well as the diffusion constant in its various phases and any other factors (*e. g.*, chemical combinations) which may affect the rate of progress.

[5] The observed curve of penetration may be of the first order, *cf.*, Irwin, M., *J. Gen. Physiol.*, 1925-28, viii, 147.

$$\frac{dx}{dt} = V_M(M_o - M_i \text{ and } V_M = \frac{1}{t} \log \frac{M_o}{M_o - M_i}$$

[6] Since this cannot alter the internal or external concentration in this case (where we assume that H^+ and A^- are the only ions present in quantity) we need not consider it here.

must collide at the surface and the situation is like that in a bimolecular process of the type

$$H_o + A_o \underset{\text{outward}}{\overset{\text{inward}}{\rightleftarrows}} H_i + A_i$$

so that the observed rate $\frac{dx}{dt}$ would be equal to the difference between the inward $(\frac{dx}{dt})_i$ and outward $(\frac{dx}{dt})_o$ rates, *i. e.*,

$$\frac{dx}{dt} = \left(\frac{dx}{dt}\right)_i - \left(\frac{dx}{dt}\right)_o$$

If M_o is constant $(\frac{dx}{dt})_i$ is constant and is proportional to the rate of progress of ion pairs through the protoplasm, (D_P), to the permeability of the protoplasm to ion pairs, (P_P), and to the number of collisions of H^+ and A^- at the external surface which in turn is proportional to the product of H^+ by A^-. Hence we may write

$$\left(\frac{dx}{dt}\right)_i = D_P P_P HA$$

According to the mass law $HA = KM_o$. Substituting this value we have

$$\left(\frac{dx}{dt}\right)_i = D_P P_P K M_o$$

In the same way we may write $(\frac{dx}{dt})_o = D_P P_P K M_i$ and the observed rate $\frac{dt}{dx}$ will be equal to $(\frac{dx}{dt})_i - (\frac{dt}{dx})_o = D_P P_P K M_o - D_P P_P K M_i$. Putting $D_P P_P K = V_P$ (the apparent velocity constant of the process) we have

$$\frac{dx}{dt} = V_P(M_o - M_i) \quad \text{and} \quad V_P = \frac{1}{t} \log \frac{M_o}{M_{i_o} - M_i}$$

At the beginning (when $M_i = 0$) we may call the rate $(\frac{dx}{dt})_{Pb}$ and write

$$\left(\frac{dx}{dt}\right)_{Pb} = V_P M_o$$

and in the same way the rate at the beginning when molecules alone enter may be called $(\frac{dx}{dt})_{Mb}$ so that we may write $(\frac{dx}{dt})_{Mb} = V_M M_o$. If both molecules and ions enter we have

$$\left(\frac{dx}{dt}\right)_{Mb+Pb} = \left(\frac{dx}{dt}\right)_{Mb} + \left(\frac{dx}{dt}\right)_{Pb} = (V_M + V_P)M_o$$

and the apparent velocity constant of the process $V_{M+P} = V_M + V_P$.[7]

[7] We assume that the ions and molecules enter without mutual interference, which seems reasonable as long as the pH value is the same in the external solution, the protoplasm and the vacuole. In case the pH value in the vacuole is less than that in the external solution some of the ion pairs will be changed to molecules on entering but the fraction thus changed will be constant as long as the pH value of the vacuole is constant.

Since $V_M = D_M P_M$ and $V_P = D_P P_P K$ it is evident that if $D_M = D_P$ and $P_M = P_P$ we have $V_P = V_M K$. If K is small V_P will be small in comparison[8] with V_M and $(\frac{dx}{dt})_{Pb}$ will be small in comparison with $(\frac{dx}{dt})_{Mb}$. In other words if the protoplasm is permeable to molecules we shall expect very little increase in the rate in case ion pairs enter in addition unless K is large. In general the penetration of strong electrolytes into *Valonia* and *Nitella* appears to be very slow and the electrical resistance of the protoplasm of these cells seems to be high, indicating that the value of D_P or of P_P is small. Thus in the experiments of Dr. Blinks on *Valonia* and *Nitella,*[9] the electrical resistance of the protoplasm in external contact with NH_4Cl is very high but we know that ammonia enters very rapidly and raises the pH value of the sap; presumably it enters as NH_3 or as undissociated NH_4OH.

If K is small we should not expect to find much difference experimentally between the velocity constants at high and low pH values in the case of a weak acid. This view is supported by the results of preliminary experiments (carried out by Mr. Jacques) on the penetration of CO_2 into *Valonia* at high and low pH values.

It is evident that even when ion pairs alone enter, the rate of penetration falls off with decrease of M_o and it must therefore fall off with increase of pH value and of A_o when the total external concentration is kept constant.

These conclusions will apply qualitatively in case the time curve is not of the first order.

In case of an exchange of ions[10] of the same sign going in opposite directions, *e. g.*, exchange of H^+ for Na^+, the total quantity of cations, Q, passing through the membrane in unit time is proportional to the total concentration $(H + Na)$ of cations inside multiplied by that outside so that we may put $Q = R(H_i + Na_i)(H_o + Na_o)$ where R is a proportionality factor and the subscripts *i* and o denote the concentrations inside and outside respectively. The

[8] In case the curve is not of the first order the velocity constants may be compared (without determining the equation of the curve) as follows: If the velocity constant of two curves (I and II) are V_I and V_{II} we may put $V_I \div V_{II} = T_{II} \div T_I$ where T_I is the time required to bring curve I to a given stage (*e. g.*, half way to equilibrium) and T_{II} is the time required to bring curve II to the same stage.

[9] For experiments on penetration of NH_2 into *Valonia* see Brooks, M. M., *Pub. Health Rep.*, Washington, D. C., 1923, xxxviii, 2074. For experiments on *Nitella* see Irwin, M., *J. Gen. Physiol.*, 1925, ix, 235.

[10] Doubtless the cell can produce sufficient ions (*e. g.*, $H^+ + HCO_3^-$) to ensure adequate exchange.

quantity of H^+ passing out is equal to $\frac{Q\,H_i}{H_i + Na_i}$ and that passing in is equal to $\frac{Q\,H_o}{H_o + Na_o}$ and the net amount passing in is the difference between these expressions: on reducing them to a common denominator we get

Net amount of H passing in $= R[(H_o\,Na_i)—(H_i\,Na_o)]$.

The same reasoning would apply to a strong electrolyte[11] such as KOH since it is permissible for purposes of calculation to assume that a few undissociated molecules are present in the case of strong electrolytes. It seems possible that K^+ and OH^- might combine at the non-aqueous surface of the protoplasm to form a molecule which could pass through and dissociate again on reaching an aqueous medium. We may perhaps use as an analogy a closed system containing 2 aqueous solutions of HCl separated by air. As HCl has some vapor pressure at higher concentrations it is evident that the air must contain some molecules of HCl (which may pre-exist as such in the solution or may result from a combination of H^+ and Cl^- at the surface to form a molecule[12] which passes into the air). The product (H) (Cl) is the same at equilibrium in the 2 aqueous solutions (if we use activities) but in the gas phase equals zero. In this respect the gas phase may resemble the non-aqueous layers of the protoplasm (except that the product in such layers might be greater than zero).

In the case of HCl the vapor pressure increases slowly with the concentration up to a certain point after which it rises rapidly. The same may be true of the non-aqueous layers of the protoplasm. Such a result has been observed,[13] "injury" being mentioned as a possible cause, which may of course be the case.

Summary: if the time curve of penetration is of the first order the rate of penetration of undissociated molecules may be regarded as $(\frac{dx}{dt})_M = D_M P_M (M_o—M_i)$ where D_M is the rate of progress through the protoplasm, P_M is the permeability of the protoplasm to molecules, M_o is the external and M_i the internal concentration of undissociated molecules. The corresponding value, when ion pairs alone enter (in the case of a binary electrolyte with no exchange of ions), is $(\frac{dx}{dt})_P = D_P P_P K (M_o—M_i)$ where K is the dissociation constant. It is evident that if the protoplasm is equally permeable to undissociated molecules and to ion pairs (*i. e.*, if $P_M = P_P$) the penetration of ion pairs will be very small when K is

[11] Osterhout, W. J. V., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 234.

[12] Since we assume that a molecule of HCl passing from the air to the aqueous surface can dissociate to form ions the reverse process must also be possible.

[13] Irwin, M., *J. Gen. Physiol.*, 1925-28, viii, 147.

small. When K is large the high electrical resistance of the protoplasm in *Nitella* and *Valonia* suggests that either D_P or P_P is small (*i. e.*, when a substance is present largely as ions there is very little penetration).

If the external concentration remains constant while the pH value changes so as to decrease the concentration of undissociated molecules the rate of penetration will diminish even when the protoplasm is permeable to ion pairs only.

These conclusions apply qualitatively when the time curve of penetration is not of the first order.

In case of an exchange of ions the rate will be proportional to the product of the exchanging ions on opposite sides.

The fact that certain weak electrolytes enter rapidly and that the electrical resistance of the protoplasm in contact with strong electrolytes is very high suggests that strong electrolytes may enter largely as undissociated molecules formed at the surface by collision of ions.

4214

Investigations of Methods in the Study of Anaphylaxis.*

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Comparisons were made of the intraperitoneal, respiratory and Dale methods with the intravenous route to determine which method of inducing anaphylactic shock was most reliable in the evaluation of a given state of hypersensitiveness. To make such a determination, we deviated somewhat from the regular method of a single shock dose and employed what might be termed the double shock method, that is we compared 2 criteria in the same animal.

In the first group we gave intraperitoneal injections to 46 sensitized animals. From one to several hours later these same animals received an intravenous injection of the same material in considerably smaller amounts.

Of these 46 animals, 21 were negative by both methods. Of the remaining, after the intraperitoneal injection 12 were negative, 3 gave a ± reaction,[1] 5 gave a + reaction and 5 gave a +++ reac-

* This work is being carried on under "The Crane Research Fund for the study of Allergic Diseases in Children."

[1] — no anaphylactic reaction. ± doubtful reaction. + showing dyspnea and

tion. After the intravenous injection 2 gave a + reaction, 5 gave a ++ reaction, 4 gave a +++ reaction and 14 died. Comparing our results, the reactions following the intravenous injection were more severe than those following the intraperitoneal injection.

In the next group we exposed 29 nasally sensitized animals to the same antigenic dust after a suitable incubation period. As in the first group, these animals were later given an intravenous injection of an extract of this antigenic dust.

Of these 29 animals, after the inhalation exposures 7 were negative, 5 gave a ± reaction, 8 gave a + reaction and 9 gave a +++ reaction. After the intravenous injection 4 were negative, 2 gave a ± reaction, 3 gave a + reaction, 3 gave a +++ reaction and 17 died. Here again after the intravenous injection more pronounced reactions were obtained than after the inhalation.

In a third group of studies we have compared the uterine horn reaction and the intravenous method in the same animal. Departing from the original method of Dale wherein he killed the animal by a blow over the head and then removed the uterine horn, we first completely anesthetized a virgin guinea pig, performed a laparotomy, and removed the uterine horn which was immediately set up in oxygenated Locke's solution and attached to a recording drum. The abdominal wound was then sewed and the animal allowed to recover completely from her anesthesia. She was then given an intravenous injection of the specific antigen to which she was sensitized. We have thus a graphic tracing of a sensitized uterus as well as a reaction after intravenous injection in the same animal.

In this group of 23 animals the Dale was positive in 11 cases, doubtful in 1, and negative in 11, whereas with the intravenous method 18 died and only 5 were negative. The 5 which were negative after the intravenous injection also showed a negative Dale reaction.

From our results we believe there is evidence that the intravenous route shows a greater reliability as a shock inducing method in comparison with the intraperitoneal, respiratory and Dale methods as a final criterion for the determination of a state of hypersensitiveness.

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scratching. ++ marked dyspnea, convulsive movements. +++ severe dyspnea, convulsions, collapse but final recovery. Death—typical anaphylactic death with completely ballooned lungs, this death occurring usually within a few minutes after injection.

4215

Source of Increase in Serum Calcium Induced by Irradiated Ergosterol.

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From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

We have shown that irradiated ergosterol is able not only to raise the level of the inorganic phosphorus and calcium of the blood, when it is decreased in rickets and in tetany, but that it brings about hypercalcemia when given to normal infants or animals.[1] The question naturally suggests itself as to whether this calcium is taken from the bones and other tissues, or is the result of increased absorption from the intestine. In order to investigate this point, rats were placed on a diet almost free of calcium and high in phosphorus, for a period of 3 days, in other words, until there was no longer any calcium within the intestinal tract. It was found that this procedure greatly reduced the calcium content of the blood, that it fell from the normal of 10 to 6.4 mg. per 100 cc. of serum. In fact, it reached about this level within 24 hours. This calcium depletion of the blood could, however, be brought about only in young animals weighing 100 gm. or less and did not develop in the adult rat. It may be added that the low calcium concentration tended to right itself and to reach the normal plane in the course of succeeding weeks.

When large amounts of irradiated ergosterol (1 mg. daily) were fed to young rats that had been prepared in this way, it was found that without exception the calcium could rapidly be increased 50% or more. This took place although the animals received approximately only 0.8 mg. Ca and 400 mg. P a day, which is a Ca:P ratio of 1:500. A series of experiments showed that the comparatively high amount of phosphorus in this ration played an important rôle, markedly interfering with the absorption of calcium. These results indicate that when the diet contains almost no calcium, the calcium which is supplied to the blood on giving irradiated ergosterol is derived from the tissues. Similar tests are being carried out with animals receiving an adequate amount of calcium.

[1] Hess, A. F., Lewis, J. M., and Rivkin, H., *J. Am. Med. Assn.*, 1928, xci, 783.

4216

Chemical and Pathological Changes in Livers of Copper-Fed Animals.

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A study of the chemical and pathological effects of copper on the liver was made on a series of guinea pigs, albino rats and rabbits. Different groups of matured Belgian hares, kept on a standard diet of hay and oats, with cabbage and carrots on alternate days were fed daily doses of various copper salts.

In each of the rabbits, yellow or yellowish brown refractile pigment was found in the liver cells. In some, pigment was also present in phagocytes in the portal areas and occasionally in connective tissue cells in the same region. In a few animals, some of the endothelial cells of the sinusoids contained pigment. Iron was not found in the pigment, which stained with fuchsin, but not with Sudan III, Scharlach R. nor osmic acid. Nile blue sulphate colored the pigment greenish blue to deep blue. The amount of connective tissue in the portal areas varied in different portions of the same sections and in different animals. It was nowhere sufficiently increased to justify a diagnosis of cirrhosis. The amount of copper in the livers showed considerable variation even within the same group, though in general the livers of those animals receiving metallic copper had the largest amounts.

Livers of rabbits dosed with sodium acetate contained pigment which had the same staining reaction as the copper fed animals. The livers of normal rabbits contained pigment and identical variation in the quantity of connective tissue in the portal areas as were observed in copper fed rabbits.

Rabbits fed carrots alone showed a marked increase in the quantity of pigment present over that present in the piece of liver removed before the animals were kept exclusively on a carrot diet. Rabbits on a carrot diet did not show an increase in the pigment when fed copper over that observed in rabbits which were kept on carrots for only 14 days. Turnip fed rabbits also showed a small amount of pigment.

The results on guinea pigs and albino rats were entirely negative. *Conclusions:* From the above experiments it has been concluded that copper does not cause pigmentation nor cirrhosis of the livers of rabbits, rats, or guinea pigs; and that the pigment found in the liver of rabbits is probably of exogenous origin.

4217

Effect of Barium Chloride and Ouabain upon the Onset of Ventricular Fibrillation.

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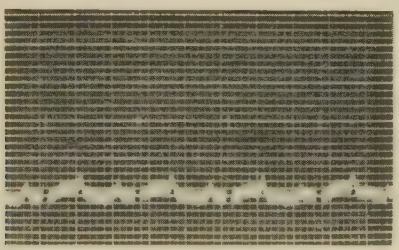
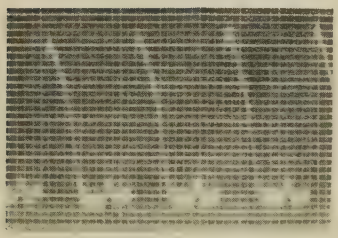
In experiments on dogs subjected to chloretone narcosis, section of the vagus nerves, artificial respiration and the exposure of the heart by removal of the sternum, in which ouabain, 1/3 lethal dose, or barium chloride, ½ mgm. per kilogram, was administered intravenously, it was observed that intense injury to the myocardium did not, as normally, induce the onset of ventricular fibrillation. Rapid excision of the entire cardiac apex, for example, caused no change in the cardiac rhythm (Fig. 1) until exsanguination and asphyxia induced heart block and the cessation of the heart beat, although ventricular fibrillation appeared under the same conditions when either of these drugs was not previously administered. They also proved to be a satisfactory aid in preventing ventricular fibril-

FIG. 1.



a



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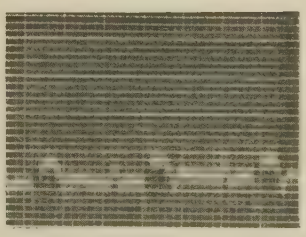

c

a—Electrocardiogram—axial lead. Time in 25th seconds. *b* and *c*—after excision of the entire cardiac apex (the lower third of the heart). *b* is the axial lead and *c* the transverse lead.

[1] Gold, H., *Arch. Int. Med.*, 1925, xxxv, 482.

lation, which is prone to occur after frequent injection of saline solution into the myocardium.

Gold[1] has found that the fatal dose of ouabain is not lessened by ligating the coronary arteries in the cat, and therefore there is no experimental evidence that an increase in the predisposition to ventricular fibrillation by the administration of the drug in the human exists when coronary artery closure has occurred. The effect reported here suggests an explanation for this fact and indicates that the action of digitalis in therapeutic doses is one which tends to prevent the onset of ventricular fibrillation when conditions predisposing to it are present, and that the administration of the drug is advisable where the onset of ventricular fibrillation is feared.

4218

Effect of Altering Venous Inflow to the Heart on the Voltage of the Electrocardiogram.

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It is known that great alteration in the heart rate tends to alter the form of the electrocardiogram; it becomes smaller with rapid rates of beating and larger when the rate is decreased. This relation between heart rate and voltage is independent of the extracardial nerves; it occurs in the denervated heart.

In the heart of the dog, exposed by removing the sternum after narcosis with chloretone, section of the vagi and the institution of artificial respiration, clamping the *vena cavae* is associated with a

FIG. 1.

a b c

Axial electrocardiogram. Time in 50th seconds. *a*—normal; *b*—effect of the rapid infusion of saline solution into the superior *vena cava; c*—effect of clamping the *vena cavae.*

decrease in the voltage of the electrocardiogram which appears immediately after the interruption of the venous inflow. When the clamps are removed the curve as rapidly returns to the original voltage, providing they have not been left on too long. This change occurs before there is any change in the rate or rhythm. Conversely, the rapid infusion of saline at body temperature into the superior *vena cava* causes some increase in the voltage of the electrocardiogram during the period in which it is flowing (Fig. 1).

These effects suggest that the volume of the venous inflow to the heart may govern the alteration in the voltage of the electrocardiogram which is associated with the following conditions: (1) that which follows changes in the heart rate, (2) that associated with hydropericardium, and (3) the phasic changes in the voltage of the electrocardiogram which are associated with the movements of respiration. The latter are known to cause rhythmic alterations in the venous inflow to the heart.

FIG. 1. Axial electrocardiogram. Time in 50th seconds. Effect of rotation of the heart upon the transverse axis of its base. With the diminution of the angle between the long axis of the heart (the electrical axis) and the plane of the Einthoven schema the voltage increases.

4219

Effect of Altering Position of the Heart on the Voltage of Electrocardiogram.

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Rotation of the heart of the dog *in situ* upon its longitudinal or antero-posterior axes alters the direction of the QRS complex of the electrocardiogram.[1] Altering the position of the heart in this manner or shifting the points of the leading about the heart as has been done by other investigators, however, maintains unchanged the relation of the heart to the plane which is defined by the points of the triangle from which the leads are taken.

Altering the long axis of the heart with reference to this plane ought to affect the voltage of the curve for the following reason: In the schema of Einthoven the heart is considered as a simple potential difference between 2 points. It is represented as the line between these points (the electrical axis). This line normally corresponds in an approximate manner to the long axis of the heart. The voltage of the electrocardiogram is recorded from the points of the Einthoven triangle, and is therefore proportional to the perpendicular projection of the line representing the electrical axis of the heart to the sides of the triangle (or to the relation between the absolute potential difference developed by the heart and the cosine of the angle between the direction in which the potential is developed and the line of lead). For similar reasons, if the heart is rotated in the plane perpendicular to that defined by the 3 points of the triangle, *i. e.*, on the transverse

[1] Meek, W. J., and Wilson, A., *Arch. Int. Med.*, 1925, xxxvi, 614.

axis of its base, the voltage registered by all 3 leads will diminish in proportion to the angle of inclination between the plane of the leads and the electrical axis. When they are parallel the voltage registered is maximum, and when they are perpendicular it is minimum, without regard to the relations already existing between them within the plane of the leading. Therefore a heart in which the electrical axis approaches the perpendicular to the plane of the 3 usual points of leading yet is otherwise perfectly normal will present very low voltage in all 3 leads. This can be illustrated by tilting the exposed heart in the dog while the electrocardiogram is written with the axial lead (Fig. 1). The presence of conditions such as this must be considered before assigning a value of significance to low voltage in the diagnosis or prognosis of heart disease.

4220

Aerobic and Anaerobic Examples of Hemolytic Bacterial Synergism.

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While studying the bacterial flora in the exudate in a case of chronic empyema of tuberculous origin 2 organisms were found which performed a function which neither could accomplish alone. One was a double-zoned *Staphylococcus aureus*. The other was a nonhemolytic diphtheroid bacillus.

On blood agar plates the colony of the double-zoned *Staphylococcus aureus* has a narrow zone of clear hemolysis about twice the diameter of the colony immediately around it and a wide zone of partial hemolysis about 8 times the diameter of the colony. Among the anaerobic bacteria this double zone is seen about certain strains of *C. welchii* while non-hemolytic strains of *C. welchii* have the outer zone only. The nature of these 2 changes in the hemoglobin is not fully understood.

While working with the culture from the pleural exudate it so happened that when the colonies were fished from the original blood agar culture to a fresh plate the diphtheroid bacillus and the double-zoned Staphylococcus were streaked side by side. After incubation, this plate showed that on the side toward the diphtheroid bacillus the outer zones of the Staphylococcus colonies were completely hemolyzed over an area very evidently under the influence of

some diffusible substance or physical force emanating from the colonies of the diphtheroid bacillus. Immediately around these colonies no change in the red cells was visible but over an area 8 or 10 times the diameter of the colony some chemical substance or physical force was operating which, whenever it came in contact with the outer zone of partial hemolysis from the Staphylococcus colonies, completed the hemolytic process not only of laking the red cells but of changing the color from red to yellow. In order that this effect might be brought out more clearly a design was made on another plate by alternately dotting with the 2 cultures. Photographs of those plates show the effect produced by these 2 organisms when in juxtaposition. It was found that control nonhemolytic colonies of several other species did not have this effect, but the diphtheroid bacillus had the same effect on the outer zone of both hemolytic and non-hemolytic strains of *C. welchii*. If the Staphylococcus was planted alone and incubated for 24 hours and the bacillus was subsequently planted on the same plate. The result was the same and *vice versa* this bears out the opinion of Castellani[1] and of Holman and Meekison[2] that when 2 organisms accomplish together what they cannot do separately one initiates the process and the other finishes it.

Some months later in culturing a specimen of surgical catgut, 2 anaerobic organisms were found which had exactly the same relationship to one another. They were a double-zoned hemolytic strain of *C. welchii* and a nonhemolytic strain of *C. oedematoides*.[3, 4] The plate after anaerobic incubation gave the same appearance as the 2 aerobic organisms did before.

Recently a third example of the same phenomenon was observed when from another specimen of catgut a hemolytic strain of *C. welchii* and a hemolytic strain of *B. subtilis* were recovered. In this case, the colony of *B. subtilis* had a narrow zone of clear hemolysis about it and an outer zone of influence not visible until it came in contact with the outer zone of the hemolytic *C. welchii* colony which it completely hemolysed.

In these 3 examples, the general principle of bacterial synergism is illustrated in a strikingly visible manner.

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[1] Castellani, A., *J. Am. Med. Assn.*, 1926, lxxxvii, 15.

[2] Holman, W. L., and Meekison, D. M., *J. Inf. Dis.*, 1926, xxxix, 145.

[3] Meleney, F. L., Humphreys, F. B., and Carp, L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 675.

[4] Humphreys, F. B., and Meleney, F. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 611.

4221

Phenomenon of Local Skin Reactivity to Culture Filtrates of Various Microorganisms.

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In previous communications a new phenomenon of local skin reactivity to *B. typhosus* culture filtrates in rabbits was described.[1] The local skin reactivity was induced by skin injections of the filtrate. If 24 hours later the filtrate was injected intravenously into the locally prepared rabbits there appeared extremely severe hemorrhagic necrosis at the site of previous skin injections 4 to 5 hours after the intravenous injection. There were observed certain features which considered together distinguished this phenomenon from the known manifestations of bacterial hypersusceptibility and the Arthus phenomenon. These features were: the local reactivity, the short incubation period necessary to induce the local reactivity, the short duration of the state of reactivity, the ability to induce local reactivity by a single skin injection, the severity of the reaction and the necessity to make the second injection of the toxic agent by the intravenous route.

The factors inducing the local skin reactivity were termed skin preparatory factors and those injected intravenously were called skin reaction factors.

The *B. typhosus* skin preparatory factors were specifically neutralized by immune anti-typhoid sera. Normal and non-related heterologous sera failed to neutralize these factors.

Further studies representing attempts to reproduce the described phenomenon with culture filtrates of microorganisms other than *B. typhosus* are reported here.

The mode of preparation of filtrates and the technique of the experiments were the same as described before.[1] The phenomenon could be reproduced with the culture filtrates of the following microorganisms: *B. coli, B. paratyphosus A, B. paratyphosus B, B. enteriditis, B. dysenteriae Y, Z, Mt. Desert, Shiga* and *Flexner, B. avicida, 5 strains of Streptococcus non-hemolyticus (gamma) isolated in this laboratory from blood cultures of acute rheumatic fever, one strain of Streptococcus viridans (alpha) isolated from a pelvic abscess, one strain of Streptococcus hemolyticus (beta) isolated from a case of mastoiditis, with Pneumococcus types I, II, and III, and*

[1] PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 560; *J. Exp. Med.*, 1928, xlviii, 267.

several strains of meningococcus. (The mode of preparation of the pneumococcus and meningococcus toxic substances was somewhat different.)*

The reactions were fully developed 4 to 5 hours after the intravenous injection. They were severely hemorrhagic and necrotic. A certain percentage of rabbits proved resistant to the phenomenon and this percentage seemed to vary with the microorganism employed.

There was no relation observed between the intensity of the erythema produced by the skin injection of the filtrate alone and the intensity of the local reaction which followed the intravenous injection of the filtrate into the same rabbits. Rabbits which showed an extensive erythema after the skin injections alone at times, remained resistant to phenomenon, and *vice versa,* rabbits in which the skin injections yielded no erythema whatsoever frequently developed locally severe hemorrhagic necrosis after the intravenous injection.

24 hour interval between the skin and the intravenous injections was sufficient for the uniform reproduction of the phenomenon with the culture filtrates of above enumerated microorganisms.

With the following microorganisms neutralization of the skin preparatory factors by homologous sera was obtained, namely: Pneumococcus types I, II and III, 3 strains of *Streptococcus non-hemolyticus* isolated from blood cultures of acute rheumatic fever patients, *Streptococcus viridans* isolated from a pelvic abscess, *B. paratyphosus A, B. paratyphosus B, B. Shiga, B. Flexner, B. dysenteriae Y, B. coli and meningococcus sera.*

Normal horse serum failed to neutralize the *B. coli, paratyphosus A, B. shiga, Streptococcus non-hemolyticus* isolated from a case of rheumatic fever (one strain), and *Streptococcus viridans* (one strain) skin preparatory factors.

Scarlet fever serum failed to neutralize the *paratyphosus A* and *B. coli* skin preparatory factors.

Studies on the effect of specific sera of other microorganisms as well as the effect of various batches of normal and heterologous sera are under way.

Work is also under progress to determine whether the described phenomenon could be advantageously applied to diagnostic, prognostic, preventive and therapeutic studies on diseases in which the above mentioned microorganisms play a pathogenic rôle and also to the studies on the etiology of certain diseases.

* This phenomenon of local skin reactivity to pneumococcus and meningococcus will receive special consideration in a future communication.

4222

Clinical Experiences with a Test for Bile Salts in Urine.

J. GERARD AND G. O. BROUN.

From the St. Louis University School of Medicine.

In a previous preliminary report[1] a test for bile salts in urine was given. The method consisted in the nephelometric determination of the cloudiness produced in bile salt containing urine by acid precipitation. Further experience with this test brought to light certain defects in the original technique. At first the urine was decolorized by the addition of charcoal. This had the obvious advantage of giving a test fluid relatively free of pigment. However it was found after repeated trials that even if the charcoal was extracted with boiling alcohol it was impossible to prevent some loss of bile salts due to adsorption on the charcoal. A further loss (probably by hydrolysis) occurred in boiling the acid alcohol urine mixture for the precipitation of albumin as described in the original technique. It was noted, however, that urine containing albumin could be acidified directly without the production of a cloud, provided it is not heated. A cloud will be produced in urine at room temperature if bile salts are present. Precipitation of urochrome by salts of various metals was also attempted and found to be impracticable as some loss of bile salts occurred in all methods tried.

Since attempts at decolorization and removal of albumin all resulted in considerable loss of bile salts, we tried to determine the clinical value of a method which would not involve loss of bile salts, although open to certain other sources of error. The following simple procedure was used. The urine to be tested is filtered. To a 5.0 cc. portion is added 5.0 cc. of normal sulfuric acid. To a second 5.0 cc. is added 5.0 cc. of distilled water. Both tubes are then compared in the nephelometer against a standard prepared as follows: A urine is secured from a normal individual which does not show a nephelometric cloud when acidified. To 4.9 cc. of this normal urine is added 5.0 cc. of normal sulfuric acid and 0.1 cc. of 1% sodium glycocholate solution. A reading for opacity is obtained both for the water and the acid mixtures of the urine under test. A difference of turbidity in favor of the acid tube was considered to indicate the presence of bile salts. Occasionally a slight negative value was obtained. Such tests were considered negative. Positive readings indicating a concentration of less than 2 mg. bile salts per

[1] Broun, G. O., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 596.

100 cc. urine were not considered significant. This method is open to criticism in that substances such as uric acid and urates may be precipitated in the presence of acid. This would sometimes give a false positive test. The clinical results do not indicate that this interference was encountered very frequently.

A series of tests were made on a number of cases of liver disease and also on cases in which there was no evidence of liver pathology. The results of these experiments may be summarized as follows: Bile salts can be demonstrated in urine where a frank clinical jaundice exists. The results are negative in most cases of liver pathology without jaundice. This robs the method of any advantage over the various tests of liver function now in use. The conditions where physical examination might leave hepatic pathology in doubt, for example cases of early cirrhosis, gall bladder disease and metastatic carcinoma, do not show any well marked increases in the concentration of bile salts in the urine. The threshold of elimination of bile salts by the kidneys seems to approximate closely that bilirubin. The clinical significance of the occurrence of both substances would therefore seem identical. The quantitation of urinary bilirubin is not difficult, so that it would be preferable as a clinical test. These facts relegate the determination of bile salts in urine to a secondary place among the tests of hepatic function.

4223

Tetany and Blood Calcium After Thyro-Parathyroidectomy in the Goat.

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Though it is a well-known fact that tetany does not often follow parathyroidectomy in herbivorous animals, no adequate explanation has been offered. Of the 2 interpretations offered, the more common is that the decomposition products of the food of herbivorous animals are less toxic than those of the food of carnivorous animals. The other is that aberrant parathyroids are sometimes found in the thymus gland of herbivorous animals. Parathyroid tetany in the goat following the removal of the thyroid and the parathyroids has not occurred as frequently as in the dog. MacCallum *et al*[1] who oper-

[1] MacCallum, W. G., Thomson, H. S., and Murphy, J. B., *Johns Hop. Hosp. Bull.*, 1907, xviii, 333.

ated on 8 goats, noted violent tetany in only 2 cases. Christens[2] observed tetany in 3 goats following thyro-parathyroidectomy.

A series of 12 goats were thyro-parathyroidectomized by removing the entire thyroid capsule with the contained thyroid and parathyroids. The blood vessels were ligated at least one inch from the thyroid capsule so that any parathyroids which might be close to the

TABLE I. *Length of life and blood serum calcium.*
Calcium determinations are expressed as mg. per 100 cc. of blood serum.

Goat number	Approx. Age Months	Blood Calcium			Survival Period Days	Cause of Death	Addenda
		Before Operation	Minimal after Operation	At Death			
1	12	9.89	9.2	8.12	9	Died during attempted thymectomy	Tremors of front and hind legs
2	12	9.23	8.93		91	Sacrificed	Bread diet for over 1 month
3	8	9.76	8.96	10.23	219	Sacrificed	Muscle tremors. Bread diet for over 1 month.
4	12	9.8	8.33	6.53	20	Pneumonia of left lung	
5	4	10.1	8.89	10.1	193	Sacrificed	Symptoms of thyroid deficiency
6	4	9.7	9.3	10.1	102	Sacrificed	Bread diet for over 1 month
7	16	9.28	7.28	7.93	84	Pneumonia	Was lactating at time of operation. Muscle tremors and chorea-like movements of the head.
8	4	10.1	8.55	7.22	18	Unknown	
9	4	10.1	9.42	10.05	83	Sacrificed	
10	6	10.5	9.54	9.5	82	Sacrificed	
11	2	10.1	8.38	9.65	74	Sacrificed	Evidence of cretinism.
12	2	9.9	8.35	10.05	74	Sacrificed	Evidence of cretinism.

[2] Christens, M. S., *Compt. Rend. Soc. de Biol.*, 1905, lvii, 335.

superior pole would be removed. The part of the thymus lying outside of the thoracic cavity was also removed in all but 2 of the animals. Half of the animals were young; the other half, young adults. Two of the former were less than 2 months old when the operation was performed.

The animals were kept on a diet of corn, oats, hay and water. All were autopsied and a careful search was made for any accessory parathyroid tissue. Blood for the blood calcium determinations was drawn from the external jugular vein. The determinations were made according to the method of Clark and Collip.[3]

The blood calcium in some of the goats fell slightly after thyro-parathyroidectomy. The fall was not to as low a level as sometimes seen in the dog. Only in one case was a blood calcium found to be below the tetany level for the dog. Two of the other animals had blood calciums which were near the tetany level. From the results as judged by the blood calciums, it seems as though we were unable to effect a complete thyro-parathyroidectomy even if we did remove a part of the thymus gland. The maintenance of the normal level of the blood calcium in the dog is certainly influenced by the parathyroids. Three of these animals were on a diet of filter paper and white bread for one month. This diet has a very low calcium content. Neither the behavior nor the blood calcium of these animals was altered appreciably by this diet.

Our results are somewhat similar to those of the other investigators who have worked on this problem. We did not see violent tetany in any of our animals, though we did observe decided muscle tremors in 3 of them.

Since various authors[4, 5, 6, 7, 8] have stated that parathyroid glands are frequently found in the thymus, we removed the part of thymus gland outside of the thoracic cavity. This, however, did not rule out the possibility of parathyroids in that part of the thymus gland inside of the thoracic cavity. Though we made an effort to remove all suspicious looking tissue at the time of operation and found no parathyroid tissue at autopsy, we feel that there must have been accessory parathyroid tissue.

Summary. In the goat the usual operation for the removal of

[3] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.

[4] Baggio, G., *Arch. Scienz. Med.*, xxxvii, 354. Quoted from Dragstedt, L. R., *Physiol. Rev.*, 1927, vii, 502.

[5] Shapiro, S., and Jaffee, H. L., *Endocrinol.*, 1923, vii, 720.

[6] Nicholas, J. S., and Swingle, W. W., *Am. J. Anat.*, 1925, xxxiv, 469.

[7] Paton, D. N., and Findlay, L., *Quart. J. Exp. Physiol.*, 1917, x, 203.

[8] Meyer, A. W., *Anat. Rec.*, 1909, iii, 272.

the thyroids and parathyroids does not result in tetany. Tetany does not supervene because the goat can maintain a normal or nearly normal blood calcium.

4224

Use of Paramecia for Studying Toxins and Antitoxins (Measles, Scarlet Fever and Diphtheria).

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At the suggestion of Dr. Hektoen the effect of bacterial toxins and antitoxins on paramecia has been studied. Others have used paramecia for testing toxic action. Hamilton[1] observed that normal human serum, at a dilution of 1:5, usually was not toxic to paramecia, while serum from scarlet fever patients was nearly always toxic (85%) and serum from pneumonia patients was toxic in 66%. Takenouchi[2] found that strong diphtheria and tetanus toxins and hemolytic staphylococcus and streptococcus culture filtrates had no effect on paramecia. Cultures of *B. pyocyaneus* caused death of paramecia, but this seemed due to alkalinity of the filtrate and not to any specific pyocyanolysin, because, neutralization of the filtrate caused the effect to be entirely lost. Philpott[3] found that virulent *B. pyocaneus* and *B. enteritidis* were toxic to paramecia. Diphtheria toxin had no appreciable effect on the division or death rate of 3 species of paramecia tested.

In testing the action of filtrates of bacterial cultures on paramecia it is necessary to grow the bacteria in a medium that is itself not toxic for the paramecia. The solutions must be isotonic with the paramecia, which according to Balbiani[1] corresponds to 0.3% solution of common salt. Crane[4] states that paramecia can live 24 hours in any hydrogen ion concentration between pH 5 to 7.6.

One percent dextrose broth, pH 7.6, made with Liebig's beef extract and Witte's peptone and containing 1% sheep blood, was found suitable for the production of streptococcus toxins and was in itself not toxic to paramecia. No sheep blood was added to the dextrose broth for the production of diphtheria toxin. The bacteria were grown 5 days at 36° C. and the cultures filtered through

1 Hamilton, A., *J. Infect. Dis.*, 1904, i, 211.

2 Takenouchi, M., *J. Infect. Dis.*, 1918, xxiii, 396.

3 Philpott, C. H., *J. Morph. and Physiol.*, 1928, xlvi, 85.

4 Crane, *J. Phar. and Exp. Therap.*, 1921, xviii, 319.

Berkefeld W filters. Antiseptics must not be added to the filtrates. Two cultures of paramecia were used in these experiments with similar results. One culture was obtained from Dr. L. H. Hyman of the University of Chicago and one culture was purchased from a biologic supply house. The paramecia were grown in whole wheat water: a few grains of wheat are added to 15 cc. of tap water in a test tube and the mixture autoclaved. The culture medium must stand open to the air several days to become contaminated with bacteria before the paramecia are added. Actively motile paramecia are essential for the test. Before dying, paramecia begin to move more slowly, rotate, become darker, extrude some of their protoplasm, and finally become motionless.

To test the toxicity of a bacterial filtrate a nearly equal part of the so-called toxin was added to a culture of paramecia in a hollow glass slide. A similar mixture of paramecia and culture medium was used as a control of the activity of the paramecia. Each specimen contained approximately the same number of paramecia. The mixtures were left in a moist chamber at room temperature and examined hourly to find when, if at all, the paramecia in the mixture with toxin were dead and showed no indication of recovering motility. A potent toxin killed all of the paramecia within 5 hours. No toxin tested was found sufficiently powerful to permit dilution. Not all of the bacteria tested produced toxins strong enough to kill paramecia during the period of the experiment.

If a bacterium produced toxin potent for paramecia, an effort was made to determine whether the toxin could be neutralized by the corresponding antitoxin. Normal and immune serum were diluted with wheat water and then to one part of the diluted serum, 4 parts of toxin were added. A similar mixture of toxin and wheat water was made. These mixtures were incubated 1 hour at 36° C. Equal parts of these mixtures were then added to the paramecium culture. The final dilutions of the serum were therefore 10 times the original dilution. If concentrated immune serum was used in a test, a concentrated serum was also used for the control.

Occasionally normal serum neutralized toxins in low dilutions and some serums were in themselves toxic in low dilutions to paramecium. Often immune serums, not toxic in themselves, did not neutralize the toxins until diluted 100 or 200 times ("prozone"?). For these reasons it was found best to dilute the normal or immune serum from 1:100 to 1:1600 or higher.

Measles Toxin. Formerly rabbits have been used in testing the neutralizing effect of anti-measles-diplococcus horse and goat serum on measles antigens. Since all rabbits do not react to measles anti-

gen and many are sensitive to horse and goat serum, it has been troublesome to find rabbits suitable for these tests. Paramecia were found to be sensitive to measles toxin, being killed in 1 to 2 hours. This toxic action for paramecia was neutralized by anti-measles-diplococcus horse or goat serum, and consequently this method is considered useful in standardizing these serums. The goat serum was not concentrated, while the horse serum was. The serums neutralized measles toxin in final dilutions of 1:1600 to 1:16,000. The unconcentrated goat serum neutralized the toxin in as high dilutions as concentrated horse serum (Table I).

TABLE I. *Action of Measles Toxin and Antitoxin on Paramecia.*

Paramecium Suspension Parts		Parts		Parts	Paramecia Living After		
					1 Hr.	2 Hrs.	3 Hrs.
5	Culture Medium,	4	Wheat Water,	1	+++	+++	+++
5	Measles Toxin,	4	" "	1	+++	0	0
5	" "	4	Normal Goat Serum 1:100,	1	++	0	0
5	" "	4	" " " 1:200,	1	+++	++	0
5	" "	4	" " " 1:400,	1	+++	+	0
5	" "	4	" " " 1:800,	1	++	0	0
5	" "	4	Antidiplococcus Goat Serum[1] 1:100,	1	+++	+++	+++
5	" "	4	" " " 1:200,	1	+++	++	++
5	" "	4	" " " 1:400,	1	+++	+	++
5	" "	4	" " " 1:800,	1	+++	0	0
5	" "	4	" " " 1:1600,	1	++	0	0

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Serum of goat immunized with coccus from measles.

Scarlet Fever Toxin. Strains of hemolyzing streptococci were tested by the opsonic method[5] to determine whether or not they were scarlatinal streptococci. For these tests unconcentrated serum from a horse immunized with scarlet fever streptococci by a modification of the Dochez method, was furnished by Dr. Benjamin White of the Laboratory of the Massachusetts Department of Health. This serum was specific for scarlet fever streptococci. Toxins were prepared with these streptococci and their effect on paramecia studied. Neutralization experiments with concentrated scarlet fever antitoxin (Dr. White) were made with toxic strains. One commercial scarlet fever antitoxin was tested also.

Eighteen strains of scarlatinal streptococci were studied, 12 were toxic, 4 partially toxic and 2 not toxic for paramecia. Four strains of hemolytic streptococci not belonging to the scarlet fever group

[5] Tunnicliff, R., *J. Am. Med. Assn.*, 1926, lxxxvi, 625; *J. Infect. Dis.*, 1928, xli, 272.

were toxic to paramecia. Two of these strains were from the blood from patients with septicemia, one from a patient with pyemia and one from the skin in erysipelas. Ten nonscarlatinal strains from patients with sore throat, acute rhinitis and *otitis media* were found to be not toxic to paramecia.

The toxins from the scarlet fever streptococci were all neutralized by the Massachusetts antitoxin in final dilutions of 1:2000 to 1:32,000 and not by the concentrated normal serum used for control. The scarlet fever toxins which were only partially toxic for paramecia were also neutralized by this serum, but experiments with such toxins do not give convincing results unless the differences between the specimens treated with normal and immune serum are clear and distinct.

TABLE II. *Action of Scarlet Fever Toxin and Antitoxin on Paramecia.*

Paramecia Suspension Parts				Parts				Parts	Paramecia Living After 1 Hr.	2 Hrs.	3 Hrs.
5	Culture	Medium,		4	Wheat	Water,		1	+++	+++	+++
5	Scarlet	Fever	Toxin,	4	”	”		1	++	0	0
5	”	”	”	4	Normal	Horse	Serum, 1:100,	1	+	0	0
5	”	”	”	4	”	”	” 1:200,	1	+	0	0
5	”	”	”	4	”	”	” 1:400,	1	++	0	0
5	”	”	”	4	Scarlet	Fever	Antitoxin[1] 1:100,	1	+++	+	+
5	”	”	”	4	”	”	” 1:200,	1	+++	+++	+++
5	”	”	”	4	”	”	” 1:400,	1	+++	+++	+++
5	”	”	”	4	”	”	” 1:800,	1	++	0	0

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Obtained from the Massachusetts Antitoxin and Vaccine Laboratory

The Massachusetts antitoxin was specific for scarlet fever streptococci and did not neutralize nonscarlatinal toxins. The commercial scarlet fever antitoxin was not specific, neutralizing not only the scarlet fever toxin, but also the erysipelas toxin and the toxins from the nonscarlatinal streptococci isolated from the blood.

Diphtheria Toxin. A filtrate of the Park-Williams No. 8 strain of diphtheria bacilli killed paramecia in 5 hours. Normal horse serum neutralized this toxin in only low dilutions, while diphtheria antitoxin neutralized it completely at a final dilution of 18,000 and partially at a dilution of 32,000 at the end of 24 hours. Eight other strains of diphtheria bacilli were tested, 7 of which were toxic for paramecia in from 3 to 5 hours. Two-tenths cc. of 5 day cultures of the Park-Williams organism and the same amount of the strain non-virulent for paramecia was injected intradermally into a normal guinea pig weighing 250 gm. In 24 hours the Park-Williams strain

produced a reddened induration, one cm. in diameter, which was followed by desquamation. The strain which was nontoxic for paramecia produced no effect in the skin of the guinea pig. This experiment indicated that the toxic and non-toxic strains acted similarly on paramecia and guinea pigs (Table III).

TABLE III. *Action of Diphtheria Toxin and Antitoxin on Paramecia.*

Paramecium Suspension Parts		Parts		Parts	Paramecia Living After		
					3 Hrs.	5 Hrs.	24 Hrs.
5	Culture Medium,	4	Wheat Water,	1	+++	+++	+++
5	Diphtheria Toxin,	4	" "	1	++	0	0
5	" "	4	Normal Horse Serum 1:200,	1	++	++	0
5	" "	4	" " " 1:400,	1	++	0	0
5	" "	4	" " " 1:800,	1	++	0	0
5	" "	4	Diphtheria Antitoxin[1] 1:200,	1	+++	+++	+
5	" "	4	" " 1:400,	1	+++	+++	+++
5	" "	4	" " 1:800,	1	+++	+++	+++
5	" "	4	" " 1:1600,	1	+++	+++	+++
5	" "	4	" " 1:3200,	1	+++	+++	++

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Each cc. contained 2000 units of antitoxin. The lowest dilution of antitoxin in the mixtures contained approximately 0.29 units of antitoxin and the highest dilution contained approximately 0.0127 unit.

Summary: These experiments show that paramecia can be used to determine toxin production of certain bacteria (diphtheria bacillus, measles diplococcus, scarlet fever streptococcus) in a crude way only because the toxins are not sufficiently potent to be diluted, but that paramecia may be helpful in determining the strength of antitoxins.

4225

Experiments in Filtration of the Virus of Avian Molluscum.*

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There are conflicting statements in the literature regarding the filterability of the virus of *Avian molluscum.* Some observers reported the presence of the virus in Berkfeld but not in porcelain

* These experiments were done in part at the Lister Institute, London, under the direction of Dr. J. C. G. Ledingham, whose advice is gratefully acknowledged.

filtrates, while some have obtained the opposite results. Others have found the virus only infrequently in filtration experiments. Findlay,[1] the latest observer, found that the virus could be passed easily through Berkfeld V filters. He used a virus of much higher titer (1:10,000) than the one employed in the experiments here reported and also used young chicks (less than a week old) as his test animals, as these were found to be more uniformly susceptible than adult hens. Mudd[2] described a method for controlling filtration technic and an arrangement of apparatus which would aid in making definite observations of the ability of a virus to pass through filters. This method was used in the present series of experiments with the virus of *Avian molluscum*.

Two filters, each unused except for preliminary pressure tests of Berkfelds V, N and W, Mandler, Seitz and plaster filters with neutral charge were used. A virus suspension in normal salt solution, centrifuged until it was almost clear, was passed through these filters. The first cc. of the filtrate was kept for animal tests, the next 10 cc. portion was discarded and the 12th cc. portion kept. A detailed record was kept of the time of filtration, maximum and minimum pressures for each portion of the filtrate, and sterility tests made of the 3 portions of each filtrate. Intracutaneous tests with 0.1 cc. of each filtrate, in duplicate, were made on the combs of adult hens, giving 4 inoculations of each portion of the filtrate for each type of filter. The unfiltered virus had a titer of 1:100 when given with this method.

In the Berkfeld W filtrations, the pressures varied from 100 to 360 mm. Hg, and the time for the 1 cc. portions to pass through 2 to 7 minutes. Virus could not be demonstrated in these filtrates. In the Berkfeld N filtrations, the pressures varied from 50 to 190 mm. Hg, and the time of filtration 2 to 3 minutes. Virus could not be demonstrated in these filtrates. In the Berkfeld V filtrations, the pressures varied from 90 to 240 mm. Hg, and the time necessary to obtain the filtrates from 1 to 5 minutes. No lesions were produced with the inoculations of these filtrates. In the Seitz filtrations, the pressures varied from 80 to 380 mm. Hg, and time of filtration from 3 to 7 minutes. In one case only the 12th cc. contained virus but this filtrate proved to be contaminated. A number of filtrations were done with the plaster filters having a neutral charge. In no case did the pressure exceed 110 mm. Hg, and the time of filtration more than 3 minutes, but all filtrates except the 1st cc. portion in one

[1] Findlay, G. M., *Proc. Royal Soc.*, 1928, cii, 354.

[2] Mudd, Stuart, PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 60.

case proved to be contaminated; nevertheless the virus could not be demonstrated in any of the filtrates.

A number of the Berkfeld filtrates were inoculated again into the combs of hens by the scratch method and no lesions appeared. Since it was possible that the virus might have been present in the filtrates in insufficient amounts to produce macroscopic lesions, hens which had been inoculated with the filtrates and had given negative results were re-inoculated with unfiltered virus and in each case typical lesions ensued showing that there was, at least, insufficient virus in the filtrates to immunize the animals.

Of several filtrations with Chamberland Ll (bis) filters, using a less diluted and more turbid suspension of the virus which had a titer of 1:1000, it was possible to obtain the virus in an uncontaminated filtrate in one instance. In this case 10 cc. of a 1:20 dilution of horse serum was passed through the filter preliminary to the virus filtration in an effort to satisfy the absorptive power of the filter as recommended by Stockman and Minette.[3] This filtrate produced lesions at a dilution of 1:100 by the scratch method. The pressure necessary for the filtration varied between 600 and 650 mm. Hg, and 90 minutes were taken to obtain 2 cc. of the filtrate. The results of this filtration agree with those of Ledingham,[4] who found that this virus would pass the same type of filter.

Nicolle and Adil Bey[5] found the virus of vaccinia to be filterable after the virus had been subjected to the action of trypsin. Findlay[1] was not able to confirm this. This method was tried in one experiment with *Avian molluscum* virus. A potent trypsin was added to 1:100 dilution of the virus and incubated at 38° for 3 hours before filtering through a Berkfeld V candle. Duplicate injections of this filtrate in a hen produced no lesions.

Levaditi and Nicolau[6] reported the successful passage of a number of viruses, including vaccinia, through collodion membranes which retained certain toxins and enzymes but passed certain antibodies. Since they did not use the virus of *Avian molluscum,* 25 filtrations of this method were tried. Membranes of nitro-cellulose were cut in discs to fit a Seitz filter. The period of evaporation and of holding the discs in water before use, varied, as did the pressure used in the filtration process. As the virus suspension contained a certain amount of hen's blood the filtrate was tested in each case against a hen's blood precipitin produced in a rabbit, for permea-

[3] Stockman, S., and Minett, F. C., *J. Comp. Path. and Therap.*, 1926, xxxiv, 1.

[4] Ledingham, J. C. G., *J. State Med.*, 1925, xxxiv, 125.

[5] Nicolle, M., and Adil Bey, *Compt. Rend. Acad. Sci.*, 1906, cxliii, 1196.

[6] Levaditi, C., and Nicolau, S., *Compt. Rend. Acad. Sci.*, 1923, clxxvi, 717.

bility of the membrane. Due to the necessary handling of these membranes, sterile filtrates were obtained in 12 instances only. The results on animal inoculations showed the virus to be present in 2 filtrates but in each of these the filtrate was not sterile and the membrane failed to retain the precipitinogen. Here again, re-inocluation with an unfiltered virus in a hen which had received 10 filtrates with negative results, resulted in typical lesions.

Summary: By following the method of Mudd it was found that the virus of *Avian molluscum* was not obtained in filtrates through Berkfeld V, N and W types, Mandler, Seitz and neutral plaster filters. In one instance it was obtained in Chamberland Ll (bis) filtrates. Adult hens were used in each case as test animals. After tryptic digestion the virus was not found in a filtrate from a Berkfeld V filter. This was also true with collodion membranes which would retain a precipitinogen.

4226

Isolation of Insulin in Crystalline Form from Fish Islets (Cod and Pollock) and from Pig's Pancreas.*

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(Introduced by John J. Abel.)

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We have succeeded in obtaining insulin in crystalline form with relative ease from the islets of fishes. These crystals are practically identical in physiological activity and in sulphur content with the crystals prepared from beef insulin.

The crystallization of insulin from pig's pancreas is more difficult. About 2 years ago Professor Abel encountered difficulties in crystallizing insulin from several of the Lilly preparations. He subsequently learned that these extracts had been made from the pancreatic tissue of both pigs and beeves. Following this observation extracts made only from the pancreas of beeves were used until the present opportunity of working with extracts made from the islet tissue of fishes[1] and from the pancreas of the pig,[2] pre-

* This investigation was carried out under a grant from the Carnegie Corporation of New York.

[1] The islet material was kindly collected for us by the General Foods Company, Gloucester, Mass.

[2] The material for this work was kindly supplied to us by the Lilly Research Laboratories, Indianapolis, Ind.

sented itself. We have now succeeded in obtaining crystalline insulin from an extract made from pig's pancreas only. The main difficulty appears to us at present to be due to the higher content of fats and lipoids in pig's pancreas. It seems advisable to use beef pancreas exclusively to readily obtain crystalline insulin, unless fish islets can be obtained in considerable amounts.

It might be added that the crystallization of the products referred to above is effected by the methods previously described in papers on insulin from this laboratory and that the methods of defatting pancreatic extracts made from the pig's pancreas are those generally used.

Further detailed studies are now in progress, and at a later date a comprehensive paper will be published.

4227

Effects of Aconitine in the Rat.

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The primary purpose of these experiments was to determine the effects of aconitine on the pulse rate of the rat. In addition the lethal dosage was determined. Commercial crystalline aconitine (Lilly) was used.

In order to reduce bodily movements the rats were given 0.6 cc. of a 15% solution of urethane per hundred gm. of body weight. After the pulse rate had become constant various dosages of aconitine were injected intraperitoneally and the heart rate taken by an ausculatory method.[1] The dosage of aconitine administered in each case is stated in terms of mgm. per 100 gm. of body weight. What was estimated to be a lethal dose of aconitine, 0.032 mgm., was administered to each of 3 rats under urethane. One rat died in 41 minutes, the second in 79 minutes, while the third lived over 120 minutes.

Ten animals were given 0.0064 mgm. Under urethane the average heart rate was 337 beats per minute. Following the administration of the alkaloid the average of more than a hundred determina-

[1] Hoskins, R. G., Lee, M. O., and Durrant, E. P., *Am. J. Physiol.*, 1927, lxxxii, 621.

tions was 334 beats per minute. There was, therefore, no significant effect of the aconitine on heart rate. Similar results were obtained with 3 rats under amytal anesthesia.

Following urethane one rat was given 0.0128 mgm. and 3 others were given 0.0256 mgm. of aconitine. The heart beat became irregular in each of these animals.

Experiments were made to determine the minimum fatal dose of the alkaloid. No anesthetic or sedative was used. Two adult rats received 0.0256 mgm. and died within 2 hours. Two rats receiving 0.0192 mgm. and 2 rats receiving 0.0227 mgm. developed serious respiratory and heart disturbances but recovered. These data indicate that 0.026 mgm. is a lethal dose for the rat, while the minimum fatal dose is approximately 0.025 mgm.

The effect of the anesthetic, urethane, on aconitine poisoning was studied by reversing the order of administration, the aconitine being given first. In 2 animals a time interval of 15 minutes was allowed between the administration of aconitine and urethane, and in 2 others the time interval was 30 minutes. The dosages were 50% more than the lethal dose without urethane. Although symptoms of aconitine poisoning occurred as indicated by respiratory and heart reactions, all of the animals recovered.

From these experiments it is evident that urethane anesthesia exerts a definite effect in preventing death from what would otherwise be lethal doses of aconitine.

4228

Precipitin Production with Phosphorised Caseinogen.

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In a recent paper, Rimington and Kay[1] suggested that an ester linkage existed in caseinogen between phosphoric acid and some other constituent of the molecule. Still later Rimington[2] attempted to find out whether or not still further quantities of phosphorus could be induced to combine with the protein. The author succeeded in phosphorizing caseinogen and sero-globulin by means of

[1] Rimington and Kay, *Biochem. J.*, 1926, xx, 777.

[2] Rimington, *Ibid.*, 1927, xxi, 272.

phosphorus oxychloride in a manner analogous to the Schotten-Baumann reaction for the benzoylation of organic substances in an aqueous solution, thus avoiding the risk of incurring destructive changes in the protein. In the case of caseinogen he found 1.77% P, corresponding to a ratio of P/N = 0.130.

Through the courtesy of Dr. Rimington we have obtained some of the native phosphorized caseinogen to ascertain whether or not fundamental changes have taken place in the immunological properties of the new product. From the standpoint of "specificity" observations of this type are of fundamental interest and the more so when destructive changes in the preparation of the protein have been greatly eliminated.

One great difficulty encountered in this study was the low solubility of the native caseinogen, the phosphorized product being much more soluble, giving a thick, viscid solution. One gram of the products as ground up in a sterile mortar and distilled water was gradually added together with N/10 NaOH until the pH was 8.0. The total volume was then made up to 100 ml. By weighing it was found that the caseinogen solution was 1 :6500. The phosphorized product, however, was readily soluble.

Nine rabbits were given intravenous injections of from 5 to 10 mls. every 3 days and the injections were continued longer in the case of the caseinogen (12 against 9). When signs of shock were observed, showing that both proteins were effective as sensitizing agents, intraperitoneal injections were given. Following the injections, a rest period of about one week was allowed before a bleeding was made and the animals were given no food for a period of 24 hours prior to the bleeding. A series of 6 animals died during the course of immunization with caseinogen.

Caseinogen induced the formation of precipitins generally of low titer, with exception of one animal, the serum of which had a titer of 1 :±6500. This reading was made by means of the ring test in Hektoen precipitin tubes after 2 hours at room temperature. Under the same conditions this serum had a titer of 1 :1000 against phosphorized caseinogen. The phosphorized caseinogen induced the formation of precipitins of higher titer than that induced by native caseinogen. The serum of one animal produced marked precipitation with phosphorized caseinogen in a dilution of 1 :64000 and slight precipitation at 1 :128000. This serum precipitated native caseinogen in a dilution of 1 :26000. Only mild skin reactions were obtained in sensitized animals and the reactions were more marked in the case of the homologous protein. Complement fixation reactions

proved to be difficult because of the high anticomplementary effect of caseinogen but this phase of the problem is being studied.

Summary: Both native and phosphorized caseinogen proved to be precipitinogenic. The reactions occur in higher dilutions when the homologous proteins are employed. Antisera against the phosphorized caseinogen precipitate caseinogen and conversely antisera against caseinogen precipitate the phosphorized caseinogen. Phosphorylation of the caseinogen does not destroy its antigenic character.

4229

Intracutaneous Vaccination of Rabbits with Pneumococcus. I. Antibody Response.

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Suspensions of heat killed pneumococci were injected into the skin of rabbits at intervals of 7 days during a period of 10 to 14 weeks. The total amount of bacterial substance injected was equivalent to and often greater than that ordinarily employed in the routine immunization of rabbits by the intravenous method. Pneumococci of Types I and III and a degraded "R" strain derived from Type II were used. The sera of the treated rabbits were tested for the presence of agglutinins, precipitins, and protective antibodies. The serum obtained from 85% of the animals immunized intracutaneously with Type I pneumococcus failed to show the presence of any demonstrable type specific antibodies. Virulent cultures of Type I were not agglutinated, nor were solutions of the specific soluble substance from organisms of the homologous type precipitated by these sera even when used in high concentrations. Only rarely did the serum confer any passive protection upon mice infected with a virulent strain of Type I, and in these instances the protective titre was low. In only 15% of animals studied was there any serological evidence of type specific response to repeated intracutaneous inoculation of Type I organisms; in these instances the presence in the serum of specific agglutinins was demonstrable only in low dilutions varying from 1:1 to 1:20.

In terms of its capacity to stimulate the formation of type specific antibodies, Type III pneumococcus is at best a poor antigen. It was to be expected, therefore, that organisms of this type, when in-

jected intracutaneously into rabbits, would fail to elicit the type specific response. Such proved to be the case. None of the rabbits treated by the skin method developed demonstrable serum antibodies against Type III. The sera of these animals failed in every instance to react with the intact S cells or with the soluble specific substance of the homologous type; they failed also to protect mice against infection with minimal doses of a virulent strain of Type III pneumococcus.

Although repeated injection of type-specific pneumococci into the skin of rabbits failed in the majority of instances to stimulate the production of type-specific antibodies, the serum of all the rabbits so treated contained in high titre the species-specific, antiprotein antibodies which agglutinate the R cells derived from all types and precipitate solutions of nucleoprotein regardless of type derivation.

Immunization of animals with R forms of pneumococci evokes the formation of the antiprotein antibodies. The serum of animals containing only these antiprotein antibodies not only fails to agglutinate S cells of the homologous type but also fails to protect mice against infection with type specific strains. In the present experiments intracutaneous vaccination of rabbits with an R strain derived from Type II gave rise to the appearance in the serum of only the antiprotein antibodies. As far as the presence of these antibodies in the serum are concerned, the results of intracutaneous vaccination of rabbits with type specific pneumococci are similar to the results obtained by direct immunization with the R variants derived from type strains.

It is of interest to note that whereas the intravenous injection of Type I invariably evokes the production of type specific antibodies, the intracutaneous inoculation of the same organisms failed to stimulate the production of these antibodies in 85% of the rabbits studied. From these results it appears that after introduction into the animal body type specific pneumococci (S forms) under certain circumstances may lose more or less completely the property of eliciting the type specific antibodies, but still retain unimpaired the property of stimulating the antiprotein antibodies, and that under these conditions, they behave antigenically as do the degraded R cells and the protein extracts derived from them. These facts are significant in interpreting the phenomenon of dissociation of the complex antigen of pneumococcus in the animal body, and in determining the character of the immune response to bacterial antigens of this order.

4230

Intracutaneous Vaccination of Rabbits with Pneumococcus. II. Resistance to Infection.

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In the preceding communication it was shown that the antibody response elicited in rabbits vaccinated intracutaneously with specific type strains of Pneumococcus is characterized by the presence in the serum of antiprotein antibodies in excess, together with a corresponding decrease or complete absence of type specific antibodies. That this change in the order of specific antibodies is related to the mode of inoculation was shown by the fact that the same strain (Type I Pneumococcus), when injected intravenously, invariably stimulates the formation of the dominant type-specific antibodies.

The present paper summarizes the results of a study of the occurrence and nature of the active resistance to infection which develops in rabbits vaccinated intracutaneously with R and S forms of Pneumococcus. Briefly, it may be stated that, following repeated skin inoculation of heat-killed suspensions of type-specific pneumococci or of the R variants, rabbits acquire a considerable degree of active immunity against infection with virulent strains of homologous and heterologous types. For example, rabbits, immunized intracutaneously with Type I may survive the intravenous injection of a dose as large as 0.2 cc. of homologous culture, the virulence of which is such that 0.0000001 cc. kills normal control animals. Similarly, rabbits immunized in the same manner to an avirulent strain of Type III may survive an infecting dose of from 0.5 to 1 cc. of a culture of the homologous type which by animal passage has been rendered so virulent that 0.001 cc. to 0.0001 cc. is sufficient to kill normal rabbits. Furthermore, animals inoculated intracutaneously with Type III become as resistant to subsequent infection with Type I as animals similarly immunized with homologous organisms. Even when an R strain of Pneumococcus derived from Type II is used for injection, rabbits acquire a high degree of resistance against infection with virulent Type I organisms. This form of acquired resistance, like that recently reported by Tillett following intravenous immunization with R pneumococci, may occur in the absence of demonstrable type-specific antibodies in the blood.

Although rabbits treated in this manner develop a solid immunity against infection with virulent strains of Pneumococcus, the serum of these animals, in the majority of instances, when used for the

passive transfer of immunity to mice, affords little or no protection whatever even against organisms of the homologous type.

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Intracutaneous Vaccination of Rabbits with Pneumococcus. III. Hypersensitiveness.

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In the preceding communications, the intracutaneous vaccination of rabbits with Pneumococcus (S forms) has been shown to give rise chiefly to the formation of the antiprotein rather than the type specific antibodies, and to the development of an increased resistance to infection with organisms of homologous and heterologous types. The present paper describes briefly the development of an altered tissue reaction to Pneumococcus and its protein derivatives in rabbits which have been inoculated repeatedly into the skin with heat killed suspensions of R and S pneumococci.

Mackenzie and Woo[1] have shown that guinea pigs, injected intracutaneously with an alkaline extract of Pneumococcus, develop an allergic reaction in the skin to the bacterial protein; Zinsser and Grinnell[2] have produced allergic sensitization to pneumococcus autolysates in guinea pigs previously injected intradermally or intraperitoneally with the same material. Bull and McKee[3] have recently shown that rabbits, after recovery from infection induced by intranasal inoculation of pneumococci, are highly skin-sensitive to pneumococcus autolysate.

The present observations were made in the course of a study of the antibody response and the immunity developed as a result of intracutaneous injection of rabbits. The intracutaneous injection in normal rabbits of 0.2 cc. of a heated vaccine, representing the bacteria from 2 cc. of broth culture, is followed by the appearance locally of a circumscribed slightly raised and indurated nodule, reddish in color, and measuring about 1 cm. in diameter. Upon repeated injection at weekly intervals the reaction changes in character; the size increases, often reaching a maximum of 4 to 6 cm. in diameter, accompanied by a spreading edema and purplish dis-

[1] Mackenzie, G. M., and Woo, S. T., *J. Exp. Med.*, 1925, xli, 65.

[2] Zinsser, H., and Grinnell, F. B., *J. Bact.*, 1927, xiv, 301.

[3] Bull, C. G., and McKee, C. M., *J. Am. Med. Assn.*, 1928, xci, 396.

coloration. The maximum reaction is generally reached after 6 to 8 injections and thereafter each successive lesion tends to become less intense but to persist longer, often breaking down with the discharge of sterile necrotic material. After healing has taken place, which often requires from 2 to 3 weeks, the animals are sensitive to the nucleoprotein and other protein derivatives of Pneumococcus when tested by the skin and ophthalmic reactions.

Sterile solutions of pneumococcus "nucleoprotein" and bacterial extracts containing the purpura-producing substance, from which the nucleoprotein had been removed by fractional precipitation, were used in the tests for hypersensitiveness. In the doses employed, the nucleoprotein fraction when injected into the skin of normal rabbits gave no local reaction, while extracts containing the purpura-producing material in about 50% of normal animals caused only a faint erythematous blushing of the skin. In the skin-vaccinated rabbits, on the other hand, these protein substances elicited an inflammatory reaction at the point of inoculation which begins to appear in from 8 to 10 hours after injection and fades after 3 days. Protein extracts containing autolytic products cause a similar but often more extensive reaction with purpuric discoloration.

In the eye test in rabbits, the cornea was anesthetized and lightly scarified by the technique described by Derick and Swift,[4] and one drop of nucleoprotein solution or purpura producing extract was instilled into the conjunctival sac. In normal rabbits this procedure causes no visible reaction. In the intracutaneously vaccinated rabbits, on the other hand, a definite reaction appears within 24 hours, which is characterized by congestion of the conjunctiva, the appearance of dilated capillaries at the sclerocorneal junction, followed by turbidity of the cornea, and occasionally by the development of pannus. The reaction may persist for from 4 to 8 days. Of 30 animals immunized intravenously with heat killed pneumococci none gave a positive eye reaction, while all of the 37 animals previously vaccinated intracutaneously reacted positively when subsequently tested with the protein derivatives of Pneumococcus by this method.

This form of hypersensitiveness appears to be limited to the protein substances of the bacterial cell, since solutions of purified type-specific carbohydrates fail to elicit the reactions in sensitive animals. In terms of bacterial specificity therefore, the reactions of hypersensitiveness are species- and not type-specific.

[4] Derick, C. L., and Swift, H. F., PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 222.

The hypersensitive state induced in rabbit by intracutaneous vaccination with R and S forms of pneumococci may persist for at least 4 months—the longest period so far tested. Moreover, it has been found, in a number of instances, that, after all evidences of the ophthalmic reactions have disappeared, the intravenous injection of nucleoprotein may cause the reappearance of the eye reaction.

A study of the development of hypersensitiveness in animals following experimental infection is in progress. The evidence at present indicates that rabbits surviving a slowly progressive but localized infection in the skin become hypersensitive to pneumococcus protein and that this state may appear as early as 12 days after the onset of the lesion.

4232

Lactic Acid Content of Blood of Trypanosome Infected Rats.

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Experimental trypanosome infection in rats presents one of the simplest pictures of a protozoan blood infection. After an incubation period of about a week (depending upon the dose injected) there is a period of uncertainty when host and parasite become alternately dominant. Then the trypanosomes become definitely established in the peripheral circulation and increase in numbers progressively until death. Parallel with the increase in the number of trypanosomes there is a progressive decrease in the number of red cells. The significant features of the infection are: (1) An increase of the trypanosomes to a constant number which seems to be characteristic of the species; (2) Anemia, which is variable, ranging between 30% and 50% of the original cell count; (3) Sudden death with symptoms of dyspnea within 10 to 36 hours after the trypanosomes have reached the maximum concentration. There are no toxic symptoms and the injection of large numbers of trypanosomes or a large amount of serum taken from an animal shortly before death do not produce any symptoms of intoxication.

This picture raised 2 problems: (1) The reason for the cessation of the multiplication of trypanosomes; was it due to an inhibiting substance or to exhaustion of the substrate? (2) The actual cause of death. If the cause of death could be ascertained in this simple type of infection, the findings might throw light on the pathology

of the disease in animals in which the course of infection is more complex and hence the cause of death more difficult to establish.

To elucidate these problems we decided to study the changes in the blood chemistry during the course of the infection. Kurt Schern[1] has shown that glucose has a protective effect on trypanosomes and that there was a reduction in the fermentable substances in the liver of trypanosome infected animals. It seemed likely, therefore, that during the infection the available glycogen and glucose reserve was used up by the trypanosomes to the point of exhaustion and that at the same time lactic acid was produced which caused progressive exhaustion of the alkali reserve; this depletion ultimately resulting in the death of the animal. Our first attention was directed to the production of lactic acid.

Rats 150 to 180 gm. in weight were infected with *Tr. evansi* and killed at various stages of the infection. Blood was taken from the carotid artery and the lactic acid content determined by the method described by Freedmann, Cotonio and Shaffer.[2] Care was taken to anesthetize the animals slowly in order to avoid a sudden increase in the lactic acid as a result of excessive muscular activity. The animals were placed under an inverted beaker containing cotton soaked with ether and left there until they were dead.

The results of one series of tests are shown in the following table. Similar results have been obtained in other series. Normal control rats were killed each time for comparison.

TABLE I.

Concentration of lactic acid in trypanosome infected rats at different stages of the infection.

Serial No.	No. of tryps. per cu. mm. blood at time of test	Lactic acid: mgm. per 100 cc. of blood		Ratio increase in lactic acid over normal control
		Infected	Control	
1	2,000	28.5	29.4	0.00
2	2,000	29.8	30.1	0.00
3	300,000	43.0	27.7	1.55
4	300,000	36.0	26.0	1.40
5	668,000	48.2	29.5	1.64
6	1,180,000	106.0	29.0	3.66
7	1,080,000	103.0	35.0	2.94

It is evident from the table that there is a progressive rise in the lactic acid concentration in the blood which runs parallel with the increase in the number of trypanosomes in the blood stream.

[1] Schern, K., *Cent. f. Bakt., Orig.*, 1926, xcvi, 356.

[2] Freedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

4233

The Thyroid and the Rate of Cell Division.

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Among the many investigations on the thyroid gland, few have dealt directly with the relation of the thyroid secretion to the rate of cell division. These few experiments, with a single exception, have been carried out on cultures of protozoa and since they have been subject to certain difficulties of control, due to the material used, the results have given rise to a rather great divergence of opinion. Nowikoff,[1] Shumway,[2] and others have found that the addition of total thyroid to *Paramecium* cultures accelerated the rate of cell division and more recently Cori[3] has reported that pure thyroxin exerts the same effect, although the acceleration is less marked. Woodruff and Swingle[4] and Torrey, Riddle and Brodie,[5] on the other hand, have found that the addition of thyroxin depresses the division rate of *Paramecia* and Torrey[6] reports that thyroxin has the same effect on the eggs of *Echinometra* and *Phallusia*.

In the present work, of which this paper is a preliminary report, the writer has undertaken to follow critically the effect of thyroxin solutions on the cleavage rate of *Arbacia* eggs. Previous work has shown that the developing eggs of *Arbacia punctulata,* properly handled, are very satisfactory for quantitative work. In each experiment, eggs from a single mature female were fertilized with sperm from a single mature male and then placed in experimental and control dishes kept side by side in a large vessel of water, so that errors due to temperature differences were eliminated. Samples of eggs removed at intervals of 1 or 2 minutes from experimental and control dishes were transferred to watch glasses containing formalin which immediately arrested development. Counts of 200 to 300 eggs were made from each sample taken. In making up the thyroxin solution, pure crystalline thyroxin (Squibb) was first dissolved in about 5 cc. of N/1000 NaOH, and then added to sea water

[1] Nowikoff, M., *Arch. f. Protistenkunde,* 1908, xi, 309.

[2] Shumway, W., *J. Exp. Zool.,* 1917, xxii, 529.

[3] Cori, G. T., *Am. J. Physiol.,* 1923, lxv, 295.

[4] Woodruff, L. L., and Swingle, W. W., *Am. J. Physiol.,* 1924, lxix, 21.

[5] Torrey, H. B., Riddle, M. C., and Brodie, J. L., *J. Gen. Physiol.,* 1925, vii, 449.

[6] Torrey, H. B., *Endocrinology,* 1928, xii, 65.

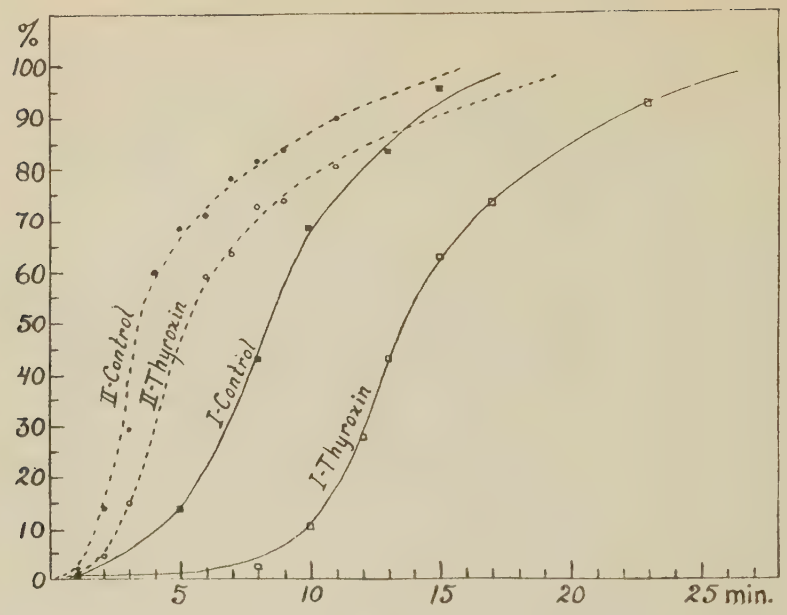

FIG. 1.

The relative effectiveness of thyroxin solutions in retarding the first cleavage of *Arbacia* eggs. Ordinate = percentage of eggs showing the first cleavage; abscissa = time in minutes. Curve *I* = thyroxin solution 1:50,000; curve *II* = thyroxin solution 1:100,000.

to make the proper concentration; in each experiment an equal amount of NaOH was added to the sea water in the control dish. In none of the concentrations used did the thyroxin change the pH of the sea water.

The relative effectiveness of thyroxin in 2 different concentrations, one of 1:50,000 and one of 1:100,000, in retarding the first cleavage of *Arbacia* eggs is shown by the curves in Fig. 1. Curves *I* show that thyroxin in sea water in a concentration of 1:50,000 retards the time of formation of the first cleavage furrow approximately 5 minutes. That this retarding action of the thyroxin persists through later cleavages has been definitely determined by counts made of later cleavage stages, and when followed through to the following day, when the eggs in the control dish had developed into free-swimming, ciliated plutei, the eggs in the experimental dish were far less developed, with no free-swimming forms and a large percentage of abnormalities.

With a thyroxin concentration of 1:100,000 the resulting retardation in the rate of cleavage is approximately half as pronounced as in the 1:50,000 solution. In the experiment represented by curves *II* in Fig. 1 the 1:100,000 concentration of thyroxin retarded the

time of formation of the first cleavage division approximately 2 minutes. Later cleavages in this experiment were correspondingly retarded, and 17 hours after the completion of the first cleavage, when virtually 100% of the control eggs had become normal, free-swimming plutei, none of the experimental eggs had progressed to the swimming stage and again there were many abnormalities evident.

In experiments in which solutions having a thyroxin concentration of greater than 1:50,000 were used the effect was correspondingly more pronounced. In thyroxin solutions of 1:25,000 for example, the reduction of the rate of division is approximately twice that produced by the 1:50,000 solution. Thus far, however, the writer has not experimented with solutions of so great a concentration that cleavage is entirely inhibited; this point is still to be determined. As would be expected, when solutions of thyroxin with concentrations of less than 1:100,000 are used the retardation is much less pronounced, until in solutions where the concentration was very slight the effect fades out altogether. In solutions of low concentration the results naturally are not so clear cut, for the eggs must be followed for several hours through several cleavages and often to the gastrula to note the retardation.

4234

Microbic Studies of Acute Infections in Animals (Albino Rat) Deprived of an Adequate Supply of Vitamin A.

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Assisted by Dorothy E. Anderson and Charles G. Blodgett.

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Previous investigators[1] have repeatedly shown that experimental animals (Albino rats), placed on diets deficient in vitamin A, develop a characteristic susceptibility to infection. Goldblatt and Benischek[2] mention that smears made of tongue abscesses in animals

[1] McCollum, E. V., *J. Am. Med. Assn.*, 1917, lxviii, 1379; Drummond, J. C., *Biochem. J.*, 1919, xiii, 95; Daniels, Amy L., *J. Am. Med. Assn*, 1923, lxxxi, 828; Macy, Icie, G., Outhouse, J., Long, M. L., and Graham, A., *J. Biol. Chem.*, 1927, lxxviii, 152; Sherman, H. C., and Burtis, M. P., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 649.

[2] Goldblatt, Harry, and Benischek, Marie, *J. Exp. Med.*, 1927, xlvi, 699.

suffering from lack of vitamin A, inevitably show a mixture of gram-positive diplococci and gram-negative bacilli.

The following communication states briefly the results observed, thus far, in a study of the bacteriology of infections resulting through vitamin A deficiency. Dr. Burt R. Shurly, Chairman of the Research Department, suggested this problem for investigation.

The bacteriological flora of the nasal cavities, posterior nasal aperture, and middle ear have been studied in both normal and infected animals. Cultures from the eye and abscesses of the upper digestive tract are also under investigation.

The experiments conducted have shown that gram-negative cocci and gram-negative bacilli are the outstanding organisms in the suppurations of the infected animals. The absence of gram-positive organisms is noticeable.

According to Gordon's[3] classification of gram-negative cocci of the nose and throat, 3 types appear to excel in the pathological animals. These are *M. catarrhalis, M. catarrhalis* subgroup A, and a microorganism classed in group 6 of the chromogenic type.

Virulence tests with young cultures of the last 2 organisms have repeatedly shown toxic effects toward rabbits, death occurring, after intravenous injections of 4 cc. of a 48-hour broth, within 24 to 48 hours.

The gram-negative bacilli from their morphological, cultural, and fermentative characteristics, appear closely related to the Friedlander-bacillus. Another gram-negative bacillus, having no action on carbohydrates, has been observed in a number of cases. A detailed study of the bacillary forms has not been made, since both types have been found in the control animal as well as in the infected animal. Virulence tests, thus far, have proved avirulent for both types of bacilli.

Young rats varying from 21 to 50 days of age were used in the experiments. Twenty-nine animals from, presumably, vitamin A-free diets and 14 animals (controls) from like diets with the addition of 5 drops of cod liver oil daily have been examined. Two experimental diets have been used. The basal rations of both diets were prepared with like ingredients, purified and formulated according to Macy and her collaborators,[1] with the exception that in one ration 15% lard was substituted for a portion of the dextrin. Due to uncertainties in regard to the vitamin A content of lard it is not assured that the fat containing ration was completely free from this vitamin. The animals were sacrificed after they had been on these diets for varying periods. A number of infected rats were

[3] Gordon, J. E., *J. Inf. Dis.*, 1921, xxix, 462.

left to succumb. The control rats were kept on the diet until all of the xerophthalmic animals had been examined. Autopsies showed that suppurations were present in the middle ear in 38%, posterior nasal aperture 58% and ethmo-turbinal area of the nasal cavities in 48%, of the 29 xerophthalmic animals. In all 85% of this group showed infection with localization of pus in one or more of these localities. In 7% cloudiness was entirely absent. Records were not obtained for the remaining animals. The control animals did not show any signs of localized pus. The middle ear, nasal cavities, and nasal apertures were clear.

Bacteriological findings for infected and control animals show that gram-negative bacilli have a higher percentage incidence in the control rats than in the infected rats. Gram-negative cocci in the control animals were found only in the posterior nasal aperture, totalling 24%. In the xerophthalmic rats the total percentage incidence of gram-negative cocci for each locality averaged 78%. Gram-positive organisms were observed in several cultures from both pathological and control animals. Of the 102 cultures from the former, one showed hemolytic streptococci, 2 showed staphylococci, and 6 showed diphtheroids. Of the 44 cultures from the latter, one showed organisms of the diphtheroid group.

These experiments were carried out during the spring and summer of 1928, extending from April to September. Mention is made of this because of the possibility of seasonal variation. Experiments under immediate investigation (October) have shown the presence of a gram-positive cocci in several animals, gram-negative cocci being absent. A report of the findings obtained during the winter months will be given in the future.

Missouri Branch.

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Auto and Homoiotransplantation of Thyroid Gland into Brain of Guinea Pigs.

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Shirai[1] and subsequently Murphy[2] have shown that it is possible to transplant successfully heterogenous tumors into the brain where they grow when after subcutaneous transplantation these transplants would have died. Murphy found the lymphocytic reaction in the brain to be lacking and he attributed the success in heterotransplantations to this factor. In the present investigations I determined how normal tissues (thyroid gland) behaved not after heterotransplantation in the brain but after homoiotransplantation, and whether the lymphocytic and connective reactions which are usually very pronounced after transplantation into the subcutaneous tissue were affected by the change in the site of the transplant. We used guinea pigs in these experiments; the transplants were allowed to remain in the host for periods varying between 20 and 120 days. I also compared the results of homoiotransplantation with those of autotransplantation.

I found that autotransplants of the thyroid gland, removed at a period varying between 60 to 120 days following transplantation, had assumed approximately the character of normal untransplanted thyroid gland. There is only the normal amount of stroma present and lymphocytes are lacking. A thin connective tissue capsule may separate the transplant from the surrounding brain tissue in which a slight increase of glia is noticeable.

Homoiotransplants removed at corresponding periods also show living thyroid tissue and in particular a number of well preserved

[1] Shirai, Y., *Japan Med. World*, 1921, i, 14.

[2] Murphy, James B., *Monographs of the Rockefeller Institute*, 1926, No. 21.

acini; however, some important differences exist between such homoiotransplants and autotransplants. In the former the preserved tissue is much smaller in amount than in autotransplants; furthermore, in the homoiotransplants the acini are more irregular in shape and size and only some of them contain colloid. The gland tissue is traversed by thick bands of dense fibrous tissue which radiate from a central core of hyaline connective tissue. The lymphocytic reaction is not pronounced, but lymphocytes in varying numbers are seen throughout the tissue and also in the surrounding host tissue. There is a marked increase in glia tissue about the transplant. At earlier periods (20-30 days) after transplantation the autotransplants appear quite similar to those seen at the later periods except that there is slightly more connective tissue present. On the other hand, the homoiotransplants in the majority of cases consist of a mass of dense hyaline connective tissue at the periphery of which a few small acini are seen; occasionally these acini contain colloid. While lymphocytes are scattered through the transplanted tissue and are also seen in the surrounding brain tissue, the lymphocytic reaction on the whole is much less pronounced than after transplantation into subcutaneous pockets.

We may then conclude that after homoiotransplantation in the brain the thyroid of the guinea pig may be found alive at periods, when after transplantation into the subcutaneous tissue it has been killed mainly through the activity of the host connective tissue and lymphocytes; but that even after transplantation in the brain the result is inferior to that obtained in autotransplantation. Furthermore, we may conclude that, whereas the lymphocytic reaction is diminished in the brain, the connective tissue reaction is at least as pronounced in this case as after transplantation into the subcutaneous tissue. It seems that at later periods a moderate new formation of acini takes place in the homoiotransplant of thyroid tissue in the brain.

4236

Effect of Graded Degrees of Heat Upon Cartilage in Homoiotransplantation and Heterotransplantation in Guinea Pig.

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In his series of transplantations Loeb concluded that the production of homoiotoxins depends upon the metabolism of the living transplants, whereas heterotoxins may be given off even by non-metabolizing, dead tissue.[1] He further had observed that through graded heating of homoio tissue it was possible to diminish its growth energy before the point is reached where the tumor cells are killed as the result of heating.[2]

In the following investigations we exposed xyphoid cartilage of the guinea pig to graded intensities of heat before transplanting it into subcutaneous pockets of other, non-related guinea pigs. Thus we tested the effect of heating on homoiotransplanted cartilage. In other experiments we treated rat cartilage in a similar way, before transplanting it into the guinea pig and thus tested the effect of heating on heterotransplanted tissue. In both series we exposed the cartilage to temperatures which in different experiments varied between 43° and 51° and the time of heating varied between 15 and 45 minutes. In every case the transplant remained in the host for a period of 20 days. In the case of homoiotransplantation we found that heating the cartilage to 47° C. for 30 minutes represents the critical point. Tissues exposed to a lower intensity of heating remained alive and elicited the typical lymphocytic reaction on the part of the host tissue; the connective tissue reaction was also present. Transplants exposed to greater intensities of heat were killed and no longer elicited lymphocytic reactions. At about the critical point the cartilage proper became necrotic after transplantation, while in a number of cases the perichondrium remained alive and produced new cartilage and the surrounding fat tissue was still preserved. At this point the lymphocytic reaction was much diminished or lacking altogether and the connective tissue reaction also was absent or much diminished. We may then conclude that in homoiotransplantation the lymphocytic and connective tissue

[1] Loeb, Leo, *J. Med. Research*, 1918, xxxvii, 353; *Biol. Bull.*, 1921, xl, 143.

[2] Loeb, Leo, *J. Med. Research*, 1902, viii, 44; *Virchow's Archiv.*, 1903, clxxii, 345; *Am. Med.*, 1903, v, 412, 1905, x, 265.

reactions depend upon the metabolism of the living tissue and furthermore that it is possible to dissociate through graded heating the activity and the life of the cartilage from that of the perichondrium.

The results are entirely different in heterotransplantation of cartilage. While here the untreated cartilage tissue may remain alive up to 28 days (Loeb and Harter[3]), even the lowest intensity of heating employed in my experiments killed the cartilage and the surrounding fat tissue. Yet the reaction of the host against the heterotransplants remained very active; lymphocytes and polymorphonuclear leucocytes as well as connective tissue surrounded and invaded the transplants and the intensity of these reactions on the part of the host were very strong. We may then conclude (1) that heterotransplanted tissue is injured through heating more markedly than homoiotransplanted tissue, owing to a summation of the injurious effects of heating and of heterotoxins and (2) that in contradistinction to homoiotoxins the production of which depends upon living, metabolizing tissue, heterotoxins may also be active even in necrotic tissue.

4237

Hepatic Lesion in Eclampsia (An Experimental Study).

WM. J. DIECKMANN. (Introduced by P. A. Shaffer.)

From the Department of Obstetrics, Washington University School of Medicine.

The hepatic lesion in Eclampsia in humans is peculiar in that it does not occur in any pregnant animal or in any other human disease. The lesion is essentially a thrombosis occurring in the capillaries of the portal vein in the periphery of the liver lobule with hemorrhage into the adjacent tissues, resulting in necrosis. It is our belief that whatever the substance is which causes this thrombosis, it must be in greater concentration in the portal system.

From clinical experience we know that curtailment of protein together with intestinal elimination will in almost all cases prevent Eclampsia. We also know that Eclampsia does not occur unless the woman is pregnant, that is, living chorionic villi must be present. The fetus may be dead for true Eclampsia has occurred with Hydatidiform Mole.

In late pregnancy placental fragments are constantly entering the

[3] Loeb, Leo, and Harter, J. S., *Am. J. Pathol.*, 1926, ii, 521.

blood stream. This placental tissue is rich in tissue fibrinogen, a blood coagulant found in tissue extracts, which must be neutralized or destroyed by substances in the blood.

Mills[1] has demonstrated that tissue fibrinogen, a cephalin-protein complex can pass through the intact intestinal wall and shorten the clotting time of the blood. If this is true it seemed to us that we could explain the production of the hepatic lesion of Eclampsia as follows: If too much of the neutralizing substance is used up by the placental tissue, it is conceivable that tissue fibrinogen from meat, by mouth, requiring the same detoxifying substance, could reach such a concentration in the portal system that thrombosis would occur.

We first wished to see if we could produce portal thrombosis. We used tissue fibrinogen prepared by the Merrel Drug Company according to Mills'[2] directions. Thus we ruled out the possible effects of other substances which are found in tissue extracts. In order to get as great a concentration as possible in the portal system we made use of the Blankenhorn cannula, through which we could daily inject tissue fibrinogen into the portal vein, at the same time injecting the same substance in the peripheral circulation, thus simulating pregnancy. Doses of 2 to 5 ml. were used peripherally and 3 to 12 ml. for the portal. Eight dogs were handled in this way, 3 of which showed lesions typical of Eclampsia. The remainder all showed marked portal vein thrombosis and hemorrhage into the liver tissue, but only to some extent from the periphery of the lobule. The fact that marked thrombosis occurred was due to over dosing resulting in coagulation of the blood in the larger branches of the portal vein, which, therefore, interfered with the production of hemorrhage. This work demonstrated that tissue fibrinogen used in this way in proper doses could cause portal coagulation and produce a liver lesion similar to that of Eclampsia.[3]

The present report is based on the combination of the peripheral injection and oral feeding of tissue fibrinogen. Seven dogs were fed fibrinogen in 3 ml. doses through a stomach tube and at the same time injected 1 to 5 ml. peripherally. These experiments varied from 3 to 7 days in duration. In 7 cases, 3 showed liver hemorrhage in the gross, similar to that in Eclampsia, 4 showed beautiful Eclamptic lesions and 6 showed portal vein thrombosis within the liver. One was entirely negative.

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[1] Mills, C. A., *Am. J. Physiol.*, 1923, lxiii, 484.

[2] Mills, C. A., *J. Biol. Chem.*, 1923, lv, 17.

[3] Reported before the American Association of Obstetricians and Gynecologists, Toronto, 1928.

The fact that limitation of protein prevents the development of Eclampsia in many cases and, furthermore, that a method of starvation and intestinal elimination gives the best results in curing the condition suggests strongly that substances which may be toxic to the pregnant woman are absorbed from the intestinal tract.

In this report we are primarily concerned with the hepatic lesion of Eclampsia; but it may be noted briefly that some of the dogs had convulsions, tonic and clonic, and some became comatose. The development of these conditions depended on the dosage. Sections of the kidney in some animals showed lesions similar to those found in humans dying of Eclampsia.

Our findings, we believe, explain in a measure why the hepatic lesion in Eclampsia can be averted by limiting the protein intake of the patient in the last months of pregnancy.

4238

Correlation between Threshold and Conduction Rate in Myelinated Nerves.

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From the Departments of Physiology and Surgery, Washington University School of Medicine, St. Louis.

In observing the effects of stimulating a nerve in the body, it is possible to lead off the cut end of the nerve into the oscillograph and thus correlate the potential form with the functional result of stimulation. Since the different fibers of a nerve are stimulated at different threshold strengths, if the difference in thresholds of different fibers were known, the oscillograph or other potential recorder could be dispensed with except for an occasional observation of threshold for the most irritable fibers, and the experimental procedure thus simplified. With this end in view, we have examined the ratios between the threshold of the first fibers stimulated in a nerve and that of other fibers, taking as criteria the thresholds of the first fibers in the various potential waves which represent fiber size groups. Gasser and Erlanger[1] have shown that the conduction rates of different fibers tend to vary as the fiber diameters, and by correlating the thresholds and conduction rates of different waves it should be possible to find the relationship between threshold and fiber size.

[1] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, lxxx, 522.

For the potential waves of 4 bullfrog sciatic nerves, the averages of the ratios of the α/β and β/γ conduction rates are 1.64 and 1.60, respectively. The same ratios for similar nerves for which the data are obtained from the table in Gasser and Erlanger's[2] paper are 1.62 and 1.60. This is approximately the ratio of fiber diameters shown in Gasser and Erlanger's charts of size distribution. The γ/δ ratios of conduction rate for 2 nerves available are 1.45 and 1.40. The ratio of average thresholds for β/α for the first 4 nerves above is 1.63, but the γ/β ratio is 2.3. No data are available for δ. The threshold appears to increase faster than conduction rate decreases; the products of these, for the starts of the α and β waves, are equal, but for the γ wave this product is 30% higher. The variations between individual nerves are within 10% of these averages.

For one vagus nerve of a cat, 425 fibers of which have been counted,* including the larger ones, the ratios for both conduction rate and largest fiber size for the starts of the first 3 main groups are about 1.6, but the thresholds increase more rapidly than the rates decrease, the product of threshold $\times$ conduction rate increasing progressively for the 9 potential waves distinguishable in this nerve. The products of threshold $\times$ rate, increase in general for both vagus and sympathetic cat nerves as the conduction rate decreases in groups of fibers of successively smaller diameter. The sympathetic is very similar to the vagus in its potential form, except that it lacks the first 2 groups of large fibers prominent in the vagus. The last 2 waves of each nerve are presumably not due to myelinated axons. The threshold of the last wave of the vagus may be 100 times that of the first. The potential waves of these nerves often appear in pairs, that is, pairs of thresholds are close together, each pair occupying about the interval of one wave in the frog sciatic. This phenomenon is similar to the frequent doubling of the β and γ of the bullfrog sciatic, which is often noticeable in nerves which have been depressed in function and whose waves, therefore, separate out because of slowed conduction.

The product of threshold $\times$ rate appears to be fairly constant for a given fiber of a nerve even with considerable depression of function, as on standing in Ringer's solution, but changes with anoxemia. It is susceptible to change with change of temperature, rising with increase. This presumably indicates that the threshold decreases proportionately less than the rate increases. The higher product, for small fibers observed, as compared with that for large, which

[2] Erlanger, J., and Gasser, H. S., *Am. J. Physiol.*, 1924, lxx, 624.

* We wish to extend our thanks to Dr. Cowdry for excellent sections of nerves made in the Cytological laboratory of the Department of Anatomy.

probably indicates that the threshold increases more than proportionately to 1/ diameter, may be correlated with the fact that, in the vagus measured, the myelin sheath is relatively thicker, in terms of percentage of total diameter, in the smaller fibers, thus offering relatively more resistance to a stimulating current. This is not inconsistent with the report of Donaldson and Hoke[3] that the axon occupies one half the total cross sectional area in different animals, our figures for the sizes of fibers which Donaldson and Hoke usually measured being 54% and 46% (for 15 and 10μ fibers) compared with their 50% average.

Qualitatively, threshold may be taken as a satisfactory criterion of which axon group is being stimulated, after a given nerve has been mapped out by means of the oscillograph, but only if the state of the nerve being stimulated is kept fairly constant, with occasional checking against the oscillograph potential record resulting from a given stimulus strength.

4239

Blood Pressure in Unanesthetized Animals Affected by "Vasopressin," Oxytocin," Pituitary Extract and Other Drugs.

CHARLES M. GRUBER.

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Recently Kamm, Aldrich, Grote, Rowe and Bugbee[1] separated from pituitary extracts what they believe to be nearly pure vasopressor and oxytocic hormones. These hormones recently were placed on the drug market under the names "Vasopressin" and "Oxytocin". Gargle, Gilligan and Blumgart,[2] Ward, Lyon and Bemis[3] have studied the effect of vasopressin upon blood pressure with uncertain results. This work was undertaken to determine if vasopressin affects the blood pressure in unanesthetized experimental animals and if so in what way does it change it.

Dogs and cats were used. In cats the operative work was done under ether anesthesia and the animals were permitted to recover. In the unanesthetized dogs local 1% procain anesthesia was used.

[3] Donaldson, H. H., and Hoke, G. W., *J. Comp. Neurol.*, 1905, xv, 1.

[1] Kamm, Aldrich, Grote, Rowe and Bugbee, *J. Am. Chem. Soc.*, 1928, l, 573.

[2] Gargle, Gilligan and Blumgart, *New England J. Med.*, 1928, cxcviii, 169.

[3] Ward, Lyon and Bemis, *Am. J. Obst. and Gynecol.*, 1928, xvi, 655.

Experiments were also performed upon dogs under chloretone anesthesia as directed by the original investigators.[1]

In animals under chloretone anesthesia vasopressin caused a rise in blood pressure although histamine produced its typical fall. In unanesthetized dogs and cats the initial injection of vasopressin caused, after a temporary slight rise, a precipitate fall in blood pressure, in some cases as much as 150 mm. of mercury. This was accompanied by a slow pulse, grouped cardiac contractions, pale, dry mucous membranes, with decreased respiration. This was followed by a prolonged rise in blood pressure, in most instances above the normal. All subsequent injections were followed either by a rise in blood pressure or no change.

In an unanesthetized animal pituitary extract caused a rise in blood pressure and oxytocin either no effect or a slight rise or fall in blood pressure.

Acetyl choline caused a decreased blood pressure and an increase after the injection of atropine in unanesthetized dogs. Vasopressin caused its typical fall in blood pressure after atropine administration. The fall in blood pressure caused by vasopressin is probably not due either to choline or histamine.

Vasopressin is far more toxic than indicated in the original communications.

Southern Branch

Tulane University, November 23, 1928.

4240

Preparation of Culture Media for Routine Cultures of Feces for Pathogenic Amebas.

CARLO J. TRIPOLI. (Introduced by F. M. Johns.)

From the Laboratory of Clinical Medicine, Tulane University of Louisiana.

As an aid to the microscopic examination of feces for the diagnosis and differentiation of pathogenic amebas, routine cultures should, theoretically, occupy a prominent place in the clinical laboratory. As described in the literature, Dobell's modifications[1] of Boeck and Drbohlav's original media[2] for the cultivation of pathogenic amebas is apparently best suited to routine use, as it furnishes a definite source of assimilable carbohydrate as food for the amebas, and further provides a mild antiseptic (acriflavine) which inhibits the growth of such frequently found bacterial flora as would preclude the obtaining of a positive culture. In obtaining and maintaining a large number of cultures of *Endameba histolytica* for the past year, many details of producing a uniformly successful medium have been studied. The following method in detail, of preparing these media has seemed to give uniform results:

I. *Ingredients of media:*

(a) Serum-Ringer solution: Beef or human blood (preferably the latter) is obtained and after clotting is placed for 24 hours in a refrigerator. The serum is pipetted off and mixed with Ringer's solution in the proportion of one part of serum to 8 parts of Ringer's solution.[3] Sterilization is accomplished by passing the fluid

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[1] Dobell, C., and Laidlaw, P. P., *Parasitology*, 1926, xviii, 206.

[2] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v, 371.

[3] Ringer's solution consists of NaCl, 9.0 gm.; KCl 0.2 gm.; $CaCl_2$ 0.2 gm. to 1000 cc. distilled water.

[4] Seitz-Wertz filters made by Empire Laboratory Supply Co., Inc., N. Y., model No. 1787/3.

through the *large type* Seitz-Wertz filter.[4] This filtered, sterile, diluted serum is pipetted off with a 50 cc. sterile pipette and placed in sterile flasks, 50 cc. to 100 cc. to each flask. These flasks are kept as cold as possible until ready for use.

(b) Starch: The best kind of starch is rice starch.[5] It possesses small grains, and is not easily hydrolyzed. This is weighed out in 0.2 gm. quantities, placed in small filter paper tubes which are made by rolling a 3½x5 cm. piece of thin filter paper about a pencil and in which the starch is kept in place by lightly crimping the ends of the paper tube. The starch in the tubes of filter paper is placed in test tubes which are then plugged with cotton and covered with several layers of wrapping paper which is well tied down over the end of the tube. The tubes are autoclaved at 15 pounds pressure for 15 minutes. After autoclaving, the paper covering is removed and the tubes are placed in a hot air oven or incubator to evaporate the little moisture that has condensed upon the walls of the tube.

(c) Acriflavine solution: A 1% solution of acriflavine[6] is prepared and sterilized in the autoclave. Acriflavine solution keeps fairly well when protected from light, preferably at a low temperature.

(d) Egg base: 4 eggs are emulsified with 50 cc. of Ringer's solution and poured into 1½x15 cm. tubes to a depth of approximately 2 cm. The tubes are autoclaved at 15 pounds pressure for 15 minutes in the upright position, which coagulates and sterilizes them, giving a flat surface upon which the amebas grow best. The pressure in the autoclave should be raised and lowered slowly in order to prevent bubbling of the media. The tubes are kept in the refrigerator until ready for use.

II. *Assembly of media:*

(a) The starch is added (0.2 gm.) to the serum-Ringer solution (50 cc.), placing the small paper tubes containing the starch into the flask. By slight agitation the paper opens up and liberates the starch. (b) Acriflavine is added by transferring 0.1 cc. of the 1% solution, which gives an ultimate dilution of 1-50,000. Some samples of acriflavine require a greater concentration (1-25,000) to retard the growth of unfavorable flora. (c) 4 to 5 cc. of sterile serum-Ringer's solution plus the starch and acriflavine are then poured into the tubes containing the coagulated egg. A preliminary

[5] Rice starch prepared by Eli Lilly & Co. (Lilly's authentic starches).

[6] Neutral acriflavine, "National," the National Aniline and Chemical Co., N. Y.

inactivation of the serum has not been found necessary. The tubed culture media should be stored in the refrigerator until used. At the time of inoculation the media should be warmed to 37° C. An equal number of tubes should be prepared omitting the acriflavine. The pH of the prepared media varies from 7.2 to 7.8 and "needs no adjustment."

III. *Method of Inoculating, Examining and Transplanting Cultures:*

A particle of fecal material about the size of a small green pea is pickel up on a wooden applicator and is transferred to a tube of plain and a tube of acriflavine charged media which have been warmed to about body temperature. With soft and liquid stools, which are more apt to contain vegetative amebas, the material used for inoculation should be obtained fresh from the patient. Proctoscopic removals, rectal washings, etc., should similarly be inoculated immediately. With formed or hard stools, which are more apt to contain cysts, the cold stool will suffice even though it be several hours old. We have obtained the same results with cysts as obtained fresh from the patients and with those several hours old.

The tubes are incubated in an upright position at 37° C.

The growth of amebas reaches its maximum in from 48 to 72 hours.

Examination of the cultures is made by skimming the debris of starch and bacteria from the surface of the coagulated egg with a capillary pipette having a large lumen (1 mm.) which has been nicked and broken with a square tip, and equipped with a Wright's rubber bulb. A drop of material is removed, placed on a slide, cover glass added, and examination made with 16 mm. objective.

To transplant the culture a drop of the material is transferred to fresh media.

Initial growth is usually obtained in both plain and acriflavine charged media. Occasionally one or the other variety only will give a positive culture.

In a large series of cultures all proven cases have given cultures of the pathogenic amebas. Non-pathogenic strains occasionally grow, but do not withstand successive transplanting in the above 2 media described. Fresh cultures should be "carefully nursed" and transplanted every 36-48 hours. Older cultures may be transplanted every 3 to 6 days.

4241

A Colorimetric Method for the Determination of Levulose in Blood and Urine.*

RALPH C. CORLEY.

From the Department of Bio-Chemistry of the School of Medicine, Tulane University.

One volume of the solution to be analyzed, a half volume of concentrated HCl, and a tenth volume of a 20% alcoholic solution of diphenyl amine, in a large test tube are heated in a boiling water bath for 15 minutes and then cooled. It has been found convenient to close the tube with a one-hole rubber stopper with the hole stuffed with glass wool. Shaking the solution with a third volume of liquid (melted) phenol causes the immediate absorption of the diphenyl amine together with the color. The addition of a half volume of 95% ethanol renders the mixture homogenous and suitable for colorimetric comparison, which may be made immediately, although this is not necessary. The color tends to darken slightly on standing. Standards are prepared similarly and simultaneously from solutions of levulose. It is felt preferable to make these latter at fairly frequent intervals from a 1% stock solution, using of course a preservative as toluene. Since 1 mg. of levulose per cc. of solution gives a fairly intense color, the standards have been made to range downwards from this concentration. Similarly if necessary the solutions to be analyzed have been diluted to within this range.

This method has been found satisfactory in the analysis of aqueous solutions, considerably diluted urines and tungstic acid blood filtrates. In more concentrated urines interference has been encountered, the color obtained being darker than it should be and frequently of a somewhat different shade. Tungstic acid filtrates contain substances that cause the development of a faint greenish blue coloration, that increases slightly the apparent color given by levulose. While theoretically dilutions and standards should probably be made from tungstic acid filtrates, as the influence is rather constant, it has been felt possible to use water, making a small frequently determined correction, without introducing significant error, except possibly when the levulose concentration is very low.

The diphenyl amine employed has been obtained from the Eastman Kodak Company. An old stock of this substance available in

* Aided by a grant from the David Trautman Schwarz Research Fund.

this laboratory was found absolutely unsuitable for this determination.

4242

Tolerance for Levulose in Several Types of Experimentally Produced Liver Injury.*

RALPH C. CORLEY.

From the Department of Bio-Chemistry of the School of Medicine, Tulane University.

In view of the unquestioned importance of the liver in carbohydrate metabolism, numerous attempts have been made to correlate hepatic efficiency and tolerance for various sugars. Among these fructose has attracted considerable attention. With experimental animals there seems but little question that impairment of the function of the liver is associated with a decreased ability to metabolize fructose, as evidenced by the greater hyperglycemia resulting. Having available a method for the determination of levulose in blood and urine, it has seemed of interest to study the effect of hepatic dysfunction on circulating levulose after administration.

In the normal rabbit, levulose disappeared from the blood in 90 minutes subsequent to its intravenous injection in doses of 2 gm. per kilo of body weight. The rate of removal was not strikingly affected by mild poisoning with various substances that are injurious to the liver, but more rigorous treatment with phosphorus, chloroform, and hydrazine sulfate did have an evident effect, levulose still remaining in the blood at the end of 90 minutes.

The oral ingestion of levulose in quantities as large as 7 gm. per kilo of body weight caused but slight increase in the total blood sugar and the appearance of very little levulose in the circulation. The liver poisons were without marked influence on the amount of levulose appearing in the blood stream, but the total blood sugar rose to very high levels when levulose was fed.

* Aided by a grant from the David Trautman Schwarz Research Fund.

4243

Infection of Cyclops with Coracidium of Oriental Diphyllobothrids and their Development to Mature Procercoid Stage.

CHI-HSIEH LI AND E. C. FAUST.

From the Parasitology Laboratories, Peking Union Medical College and College of Medicine, Tulane University.

Janicki and Rosen[1] showed that certain copepod crustaceans belonging to the genera Cyclops and Diaptomus were the first intermediate hosts of *Diphyllobothrium latum.* Okumura[2] found that *Cyclops leukarti* was the necessary first intermediate host of a species of Diphyllobothrium which he designated as *"Sparganum mansoni".* Since considerable doubt has been indicated as to whether the spargana found in man and other vertebrates in the Orient belong to one of several species, the present writers have undertaken to study the life cycle of forms found in North China.

In one series of cases eggs from naturally infected dogs and cats have been utilized as the starting point of the experiment; in another, spargana commonly found in the somatic musculature of the hedgehog, *Erinaceus dealbatus,* have been fed to uninfected dogs and cats, resulting in the development of these larvae into adult worms, from which, eggs were readily obtained. Morphological study of the mature worms in these cases has shown that the former type belongs to the species *Diphyllobothrium decipiens* (Diesing), while the latter is *D. erinacei* (Rudolphi). The eggs were washed and incubated at temperatures from 15° to 35° C. Both species developed equally well. At the lower temperatures 21 to 30 days were required, while at 35° C. only 9 days for complete development and hatching. Under favorable conditions about a 90% hatch was obtained. Once the opercular caps of the eggs had opened, the ciliated hexacanth embryos (coracidia) all emerged in about an hour. Agitation of eggs containing fully mature embryos resulted in a slightly earlier hatching.

Eight species of Cyclops were obtained in considerable number from the ponds, rice-fields and streams in the environs of Peking. None of these were ever found naturally infected with tapeworm larvae. The Cyclops were introduced one at a time into watch-glass cultures containing numbers of free-swimming coracidia and usually began immediately to ingest the coracidia, which showed no avoiding

[1] Janicki, E., and Rosen, F., *Bull. Soc. neuchateloise Sci. nat.,* 1917, xlii, 19.
[2] Okumura, T., *Kitasato Arch. Exp. Med.,* 1919, iii, 190.

reaction. In 15 minutes such a Cyclops might swallow 20 to 60 larvae, which packed the whole digestive tract. Meanwhile the ciliated embryonic membrane had been cast off by the larvae, which then proceeded to excavate a hole in the wall of the digestive tube of the Cyclops, utilizing its 3 pairs of hooks for this purpose. In 25 minutes some of the onchospheres had worked their way through into the body cavity, but a longer time was frequently required. The several species of Cyclops showed different resistances to the injuries produced in their intestinal wall, some succumbing in 2 or 3 days, others harboring as many as 8 larvae for 3 weeks or more after infection. In controlled experiments a single coracidium swimming about in a watch glass was never successful in producing an infection in a Cyclops (species A) introduced into the medium, although where large numbers of larvae were utilized the same species of Cyclops ingested the entire number. Under similar conditions species B and C ingested 70% of the coracidia, species F, 20%, and species H, 30%, while species D only swallowed 5% or less of those in the container.

The percentages of ingested larvae which arrived in the body cavity were as follows: Species A, (36); B, (26); D, none; F, (3); G, (5.5) and I, (28.5). No essential differences were found in the susceptibility of these several species of Cyclops to infection with the coracidia of *D. decipiens* and *D. erinacei*. In the body cavity of the Cyclops the onchosphere metamorphosed into a procercoid, which was characterized by having a spinose integument, with a special spinose armament around the anterior end, a complex of histolytic glands opening into the anterior end, excretory system with exact flame-cell multiples, and calcereous bodies. Meanwhile the original 3 pairs of spines of the onchosphere had become oriented posteriorly in the cercomer, which gradually became constricted off and degenerated as the procercoid approached maturity. With the exception of species D, mature procercoids were obtainable experimentally in all species of Cyclops tested, although species A produced the highest percentage. This optimum host-parasite relationship was not correlated with size, since certain small species of Cyclops harbored larger numbers of mature procercoids than did large Cyclops.

4244

Infection Experiments in Man and Other Mammalian Hosts with Sparganum Stage of Oriental Diphyllobothrids.

E. C. FAUST.

From the Parasitology Laboratory, College of Medicine, Tulane University.

The Sparganum larva of a diphyllobothrid (psaudophyllidean) cestode was first recovered from man in 1882 by Patrick Manson, who obtained a dozen of these ligulate worms from the perirenal fat of an Amoyese. The same year Scheube obtained similar material from a Japanese subject. Following these early cases numerous additional human infections were described from Japan and French Indo-China, the latter being almost if not entirely from the orbit. A few human cases have also been reported from Australia. These larvae have been consistently designated as *"Sparganum mansoni"*, after their discoverer. Meanwhile larvae indistinguishable from those recovered from the human host have been obtained from numerous vertebrates in the Orient, including frogs, snakes, birds and mammals. These, too, have commonly been referred to as *"Sparganum mansoni"*, although experimental feedings to dogs and cats of the larvae from human cases in Japan (Okumura's[1] material) have shown that the adult worms belong to 2 previously described species, *Diphyllobothrium decipiens* and *D. cordatum,* while similar experiments in French Indo-China (Joyeux and Houdemer[2]) have produced adults of another species which these investigators have described as *"Diphyllobothrium mansoni"*.

During a period of more than 8 years the writer has collected adult diphyllobothrids from naturally infected dogs and cats and their wild relatives in China and has had placed in his hands for study a complete worm of the generic group from the intestine of a native Chinese in Shanghai. These Chinese worms have been diagnosed as belonging to the following species: *Diphyllobothrium cordatum* (Leuckart, 1863), Stiles and Hassall, 1926, (dog, Peking and Amoy); *D. mansoni* (Cobbold, 1883), Joyeux, 1927, (cat, Canton, Foochow, Peking); *D. decipiens* (Diesing, 1850), Chandler, 1925, (cat, wild-cat, leopard, Peking); *D. ranarum* (Gastaldi, 1854), Meggitt, 1925, (cat, Peking); and *D. houghtoni* n. sp., (man, Shanghai; cat, Peking; dog, Wuchang). The writer has also found heavy sparganum infections in frogs, snakes and several

[1] Okumura, T., *Kitasato Arch. Exp. Med.*, 1919, iii, 190.

[2] Joyeux, Ch., and Houdemer, E., *Ann. Parasitologie*, 1928, vi, 27.

species of mammals, from representative areas in North, Central and South China. Since these larvae were indistinguishable from one another, feeding experiments have been undertaken to determine the adult species to which the larvae belong and also to find out what mammalian hosts are susceptible to intestinal infection with these spargana. The larvae were fed each in a small gelatin capsule and the feces of the animal examined systematically for *Diphyllobothrium* eggs. In a susceptible host these appeared from 2 to 3 weeks after the feeding but in negative individuals search was continued for 3 months. In non-human members of the series the animal was autopsied within 3 months or less. The results of these experiments are set down in Table I.

TABLE I.
Showing the results of feeding Oriental spargana to mammals.

Sparganum	Host	Mammal submitted to infection	Result	Species of adult worm
A	*Rana esculenta*	cat	positive	*Diphyllobothrium mansoni*
A	*Rana esculenta*	man	negative	
B	*Microhyla sowerbyi*	cat	positive	*D. decipiens*
B	*Microhyla sowerbyi*	man	negative	
C	*Canis procyonides*	cat	positive	*D. ranarum*
C	*Canis procyonides*	man	negative	
D	*Erinaceus dealbatus*	cat	positive	*D. erinacei*
D	*Erinaceus dealbatus*	dog	positive	*D. erinacei*
D	*Erinaceus dealbatus*	rabbit	negative	
D	*Erinaceus dealbatus*	man	negative	

Examination of the data shows consistently that in the 4 species diagnosed man is in no instance a susceptible host, although cats (and dogs) are infectable. Furthermore, in no member of the series was there any evidence that the spargana worked their way through the intestinal wall of the mammal and developed a somatic sparganosis.

During the course of investigating diphyllobothrid infections in Foochow, China, 2 cases of human sparganosis were discovered by Dr. Horace E. Campbell. In the one instance the infection was in an abscessed thumb, while in the other it was associated with an ulcerated wrist. Eight larvae were said to have been removed from the former infection and 10 larvae from the latter. In both cases, as in others in the locality, the patients had applied "split" frogs to the abscessed members, and since these frogs are almost always infected with the sparganum, it seems highly probable that they were the source of the infection. This evidence corresponds to that secured by Joyeux and Houdemer (1925) from patients suffering

from ocular sparganosis in French Indo-China, where a like custom is practiced to "relieve" severe conjunctivitis. To test out the assumption that any sparganum will infect the orbit the writer applied spargana of *D. erinacei,* freshly obtained from the hedgehog in Peking, to the conjunctiva of a dog, and was able to observe the invasion of the larvae through the conjunctival membrane and the development in this animal of a severe inflammatory process, which continued for several days, when the dog was sacrificed. Other experiments in susceptible hosts such as the dog indicated that the spargana when introduced aseptically into the thigh muscles were able to multiply asexually over a period of several months. In rabbits in which a similar experiment was performed no increase was observed although the original number of worms was recovered after 5 months.

These series of experiments show definitely that there are several species of spargana in China, just as in other parts of the Orient, that the cat and the dog and their wild relatives are the natural hosts of the adult worms, and that man, except in the case of *Diphyllobothrium houghtoni,* is not subject to infection with the adult worm. Furthermore, the natural hosts of the spargana are a wide variety of vertebrates, which presumably become infected from ingesting the infected first intermediate host of the worms (Cyclops). Although the evidence is not conclusive it favors the view that human sparganosis results from applying frogs infected with these spargana to inflamed and ulcerated areas of the body.

4245

Vitamin A and B Content of Canned Sweet Potatoes.

S. N. BLACKBERG. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, Tulane University.

Although sweet potatoes are an important constituent of American dietaries, information regarding their vitamin content is almost entirely lacking. With but the one exception,[1] dealing with vitamin C, no reference was found to any work on the vitamin content of this food.

The sweet potatoes used in these experiments were a yellow variety which were commercially canned in southern Louisiana, pur-

[1] Peck, E. C., *China Med. J.,* 1924, xxxviii, 125.

chased on the open market. The rats used were young, vigorous, normally growing litter-mates of the uniform Wistar Institute strain.

The plan of experimentation was to feed the negative control groups the following diets:

Lacking vitamins A and D:

Casein	20 parts
Starch	68 "
Adequate mineral mix	4 "
Agar-agar	2 "
Dried yeast	6 "

Lacking vitamin B:

Casein	20 parts
Starch	72 "
Adequate mineral mix	4 "
Agar-agar	2 "
Cod liver oil	2 "

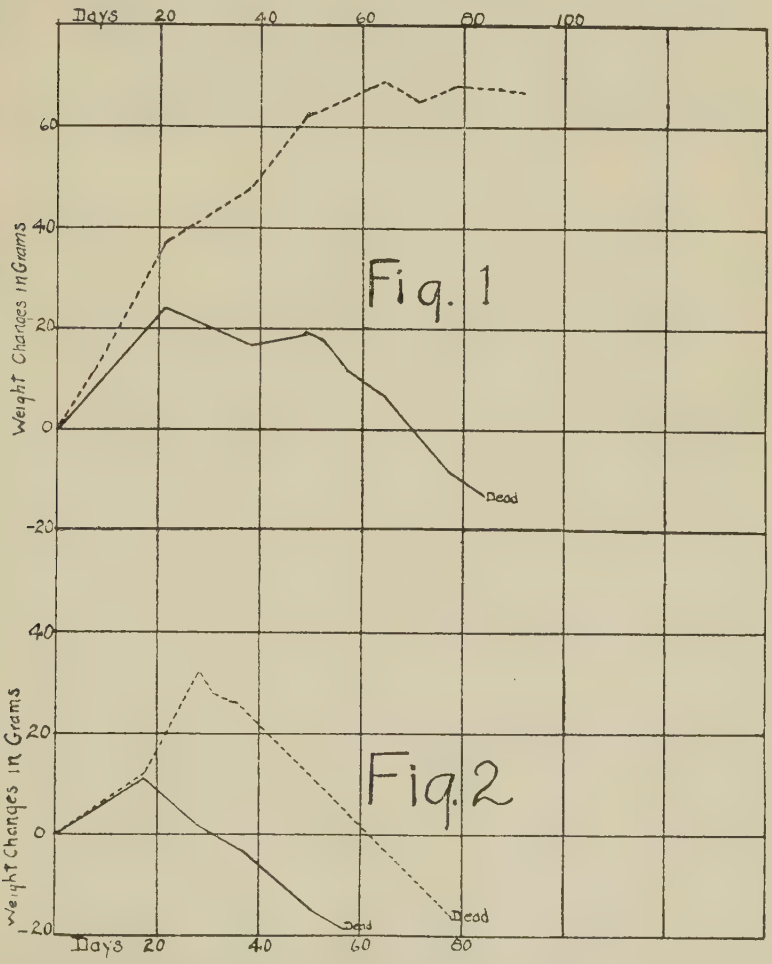

FIG. 1. Dotted line - - - - - - - average growth of curve of rats receiving vitamin A only in the tested sweet potatoes. Continuous line ———— average growth curve of rats receiving no vitamin A.

FIG. 2. The same as in Fig. 1, for vitamin B.

For the test groups 25 grams of the starch was replaced by an equal weight of the canned sweet potatoes. No attempt was made to differentiate between the 3 components of vitamin B, or to ascertain the influence of the antirachitic factor apart from that of vitamin A. The results of such differentiation will be reported at a later date.

As shown by the average growth curves of the various groups, when canned yellow sweet potatoes constituted 25% of the weight of the total diet, the vitamins A D present were sufficient to induce a much more rapid and prolonged rate of growth in the rats than did the diets lacking these vitamins but otherwise adequate. The vitamin B present also produced a distinct prolonged rate of growth, but much less than occurred in the vitamin A experiments.

These findings indicate the presence of an abundance of vitamin A and a small amount of vitamin B in the canned sweet potatoes tested.

Minnesota Branch.

University of Minnesota Medical School, November 28, 1928.

4246

Microscopic and X-Ray Investigations on Calcification of Tissue.

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From the Section of Biophysics, Mayo Clinic, Rochester, Minnesota.

An attempt has been made to determine exactly the nature of the solid inorganic phase in several types of calcified tissues, including normal bone, dental enamel, dentine, and in pathological conditions such as rachitis, salivary calculus and calcified tubercular lung tissue. Measurements were made of the mean index of refraction, n, by the oil immersion method of Becke as used by mineralogists in identifying rock constituents. The crystals present are too small to show characteristic cleavages or angles but are seen to be present in small particles apparently encased in a thin organic sheath of low refractive index. The smaller the relative amount of organic matter, the higher is the refractive index: dental enamel, which is the maximum of this group ($n = 1.62$), has only 1 or 2% of organic material. Thus the index of refraction may be taken as a measure of the degree of calcification. The following are typical values of refractive indices: human cheek-bone 1.561, dentine 1.577, enamel 1.625, rachitic rat bone 1.560, salivary calculus 1.563, and calcified lung 1.585, ± 0.003. The upper limit, 1.625, which comes the nearest to representing pure inorganic crystals, is practically the index of refraction of the minerals of the apatite series $3Ca_3(PO_4)_2.CaX_2$, where X_2 may be CO_3, O, $(OH)_2$, F_2 or SO_4. Typical minerals of this formula are fluorapatite, dahllite and podolite. We are, therefore, of the opinion that apart from the variability of X_2 the crystal phase is essentially the same in all the cases of calcification examined. No evidence was found for either $CaHPO_4.2H_2O$ or the so-called tri-calcium phosphate, $Ca_3(PO_4)_2$. The latter is unknown as a mineral and has never been prepared in a pure state in the laboratory.

X-ray diffraction patterns by the powder method were obtained for natural apatite (free from iron), and for dental enamel, normal bone, salivary calculus, tubercular calcified lung and synthetic "tri-calcium phosphate." The "tri-calcium phosphate" which we prepared had an index of refraction 1.628 and, therefore, is to be assigned to the apatite group. While there are minor differences, the patterns are very similar with respect to both position and intensity of lines. Thus they indicate similarity of crystal structure, corroborating the optical evidence.

We wish to emphasize the point that the conditions for precipitation in the bone and elsewhere will be governed by the solubility relations of these apatite minerals (such as $3Ca_3(PO_4)_2.CaCO_3$) rather than by $Ca_3(PO_4)_2$, to which much attention has hitherto been given.

4247

Antagonization of Anesthetic Effect of Magnesium Sulphate by Chlorides of Potassium, Rubidium and Sodium.

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From the Department of Pharmacology, University of Minnesota Medical School.

Meltzer and Auer in 1905 reported that subcutaneous or intravenous injections of magnesium sulphate would completely anesthetize animals, and that they could be instantly and completely aroused from this anesthesia by intravenous injections of $CaCl_2$ but not by $SrCl_2$. They made no mention of the effects of the monovalent cations. In this series of experiments we have anesthetized rabbits intravenously with m/6 $MgSO_4$ (5.5 to 11.6 cc. per kg.) solution. The controls recover equilibrium and voluntary movements in 7 to 12 minutes. Eleven rabbits completely anesthetized with $MgSO_4$ recovered equilibrium and power of voluntary movement in from 30 seconds to 2 minutes when injected with a mixture of 9 volumes m/6 NaCl + 1 volume m/6 KCl; and 2 rabbits recovered in 30 seconds and 1¼ minutes respectively after receiving 20 cc. m/30 KCl alone. Two rabbits which received 20 and 25 cc. m/30 RbCl recovered in 1 minute and 1¾ minutes respectively. Rabbits also recovered when NaCl alone was used, but much larger amounts (30-40 cc. m/3 NaCl) had to be used, and the recovery obtained in 1¼ to 2 minutes was less complete than with potassium or rubidium for though the animals would sit up, they did not regain

the use of their hind legs for quite a time after the injection. With 2 animals in which lithium chloride was used, the results were doubtful, one recovering equilibrium in 4 minutes after receiving 40 cc. LiCl m/6, the other only reacted to prodding after 6 minutes, in spite of receiving 20 cc. LiCl. Ammonium chloride, with and without sodium chloride, killed 3 animals with convulsions without any sign of their regaining consciousness.

From these experiments it is obvious that sodium potassium and rubidium ions antagonize the narcotic effects of Mg ions, in accordance with their position in the Hofmeister scale, and their effects upon surface tension and emulsions.

4248

Clinical Studies on Cardiovascular Response to Adrenalin Administered Subcutaneously.

JULIUS JENSEN. (Introduced by F. H. Scott.)

From the Department of Medicine, University Hospital, Minneapolis.

One cc. of adrenalin hydrochloride was injected subcutaneously on 19 subjects, 12 of whom were suffering from hypertension. The injections were repeated 1 to 4 times at intervals varying from 2 hours to 1 month.

Normal individuals gave a reaction to first injection characterized by a fairly rapid rise of arterial pressure which lasted 40 to 60 minutes. On a second injection the rise of pressure was much more sudden, but the contrast between the first and subsequent tests was much less marked than in the hypersensitives described below.

It was found that 8 of the hypersensitives on the first reaction presented a slight increase in the systolic blood pressure. On repetition of the injection at least 12 hours later a sudden and severe increase was noted, forming a marked contrast to the slight response on the first occasions. Four hypersensitives gave on the first tests a response resembling that given by the other 8 on second tests.

Two hypersensitives who were again examined in 2 weeks and 1 month respectively, after the first injection did not now show the sudden increase previously observed, and on repetition of the test in 24 hours it was not reproduced.

The diastolic blood pressure in all cases showed a tendency to decrease, except in cases of sudden and intense increase of the systolic pressure, when a slight diastolic increase would occur. Otherwise

the diastolic pressure was not affected by the phenomenon observed above.

The observations on the rate and rhythm of the heart agreed with those of previous observers.

This work was done under the direction of Dr. Henry Ulrich.

4249

Experimental Tularemia in Ring-Necked Pheasant.

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From the Department of Bacteriology and Immunology, University of Minnesota, and the Division of Preventable Diseases, Minnesota State Board of Health.

In his original description of the disease which he called tularemia, McCoy[1] considered the possible susceptibility of birds to this disease. Included in the list of species which he tested for susceptibility to the disease was the common pigeon. In all, he inoculated 4 pigeons with the spleen of guinea pigs dying from tularemia. He reported that the birds remained alive and apparently well.

In a previous paper[2] we reported that the ruffed grouse is very susceptible to tularemia. In a later report[3] it was stated that a varying susceptibility to this disease had been found in a number of other species of bird which had been tested. Further studies have been carried out on the degree of susceptibility of the ring-necked pheasant to experimental tularemia.

Pheasant No. 1 was inoculated in an open abrasion through the skin of the back, with heart's blood from a grouse dying of experimental tularemia. A control guinea pig inoculated with the same material died on the 3rd day with enlarged glands, and spleen and liver typical of tularemia. The pheasant remained apparently well, and was chloroformed on the 27th day. Its blood showed agglutination for *Bact. tularense* in a dilution of 1:10 and above. No scar was discernible on the back at the site of inoculation. Necropsy showed internal organs normal. Three guinea pigs were inoculated from pheasant No. 1 as follows:

No. 1. Spleen. Killed on 17th day. Normal. No. 2. Liver.

[1] McCoy, Geo. W., *Pub. Health Bull.* No. 43, April, 1911.

[2] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 515.

[3] Green, R. G., Wade, E. M., and Kelly, W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 637.

Died on 8th day. No findings of typical tularemia. Its liver and spleen were inoculated into another guinea pig, which showed no evidence of tularemia.

Pheasant No. 2. Inoculated in an abrasion through skin of back with the spleen of a guinea pig dead of tularemia. Did not develop any apparent symptoms of disease. Was chloroformed on the 12th day. Agglutination test was negative at the time of inoculation and negative just before death in a dilution of 1:10 and above. Abrasion of the skin perfectly healed. Slight superficial redness at inoculation site on under side of skin. Internal organs appeared to be normal. Guinea pigs were inoculated with tissue as follows:

No. 1. Heart's blood. Chloroformed on 13th day. Normal. No. 2. Liver. Chloroformed on 14th day. Normal. No. 3. Muscle from inoculation site. Chloroformed on 13th day. Normal.

Pheasant No. 3. Inoculated in abrasion through skin on back with spleen of guinea pig dead from tularemia. Pheasant appeared to be perfectly well until it was chloroformed on the 13th day. Inoculation wound perfectly healed. No infiltration. Internal organs appeared to be normal. Agglutination test was negative at the time of inoculation and negative at time of death at dilutions of 1:10 and above. Three guinea pigs inoculated respectively with blood, liver and muscle from inoculation site remained well. Were chloroformed on the 14th day and found to be normal.

Pheasant No. 4. Inoculated subcutaneously and intramuscularly with emulsion of liver of a guinea pig dead from tularemia. A control guinea pig died on the 4th day with inguinal glands and spleen typical of tularemia. The pheasant remained apparently well and was chloroformed on the 26th day. Its blood did not agglutinate *Bact. tularense* at a dilution of 1:10 and above. A superficial but extensive infiltration was present at the site of inoculation. The lesion was dry, hard, located just under the skin at the site of inoculation and extending into the muscle. Necropsy showed internal organs to be normal in appearance. Guinea pigs were inoculated with tissue as follows:

No. 1. Spleen. Chloroformed on 17th day. Normal. No. 2. Liver. Chloroformed on 17th day. Normal. No. 3. Muscle. Died on 4th day, showing a pneumonia. Some of the injected material still present under skin. Spleen of this pig inoculated into another pig did not produce tularemia.

Six pheasants were then utilized to study further the effect of intramuscular injection of infective material. Pheasants Nos. 5 to 10, inclusive, were inoculated into the breast muscle with ½ cc. of the same emulsion of spleen from a guinea pig dead of tularemia.

The blood of each pheasant at the time of inoculation failed to agglutinate *Bact. tularense* in a dilution of 1 :10 and above. A control guinea pig inoculated with the same material died on the 3rd day.

Pheasant No. 5. Appeared to be perfectly well the following day and was chloroformed 24 hours after inoculation. There was slight, soft infiltration at inoculation site. Internal organs appeared normal. Three guinea pigs were inoculated with tissue as follows:

No. 1. Blood. Remained well. Chloroformed on the 15th day. Found normal. No. 2. Liver. Died on the 3rd day, showing exudate over abdomen. No gland involvement. Liver and spleen normal. Exudate inoculated into another guinea pig which died on the following day showing exudate over abdomen. Exudate inoculated into 3rd guinea pig in series, which remained well and did not show lesions of tularemia when it was chloroformed on 9th day.

No. 3. Breast muscle. Died on 5th day showing enlargement of both inguinal glands. Spleen and liver typical of tularemia.

Pheasant No. 6. Appeared to be perfectly well when chloroformed on 4th day. Moderate, soft infiltration of muscle at inoculation site. Internal organs appeared normal. Guinea pigs inoculated with tissue as follows:

No. 1. Blood. Remained well. Chloroformed on 14th day. Normal. No. 2. Liver. Became sick and died on the 9th day. Both inguinal glands enlarged. Spleen and liver typical of tularemia. No. 3. Breast muscle. Became sick and died on 6th day. Both inguinal glands enlarged. Spleen and liver typical of tularemia.

Pheasant No. 7. Showed no symptoms of disease. Was chloroformed on the 7th day. Questionable infiltration at inoculation site. Internal organs appeared normal. Agglutination test on 7th day positive in a dilution of 160 complete. Guinea pigs were inoculated with tissue as follows:

No. 1. Breast muscle. Died on 7th day. Right inguinal gland enlarged. Spleen and liver typical of tularemia. No. 2 remained well. Chloroformed on 15th day. Normal. No. 3. Liver. Remained well. Chloroformed on 15th day. Normal.

Pheasant No. 8 remained apparently well. Chloroformed on the 11th day. Slight redness at site of inoculation. Internal organs appeared to be normal. Agglutination test showed complete agglutination in a dilution of 1 :320. Three guinea pigs inoculated respectively with blood, liver and breast muscle remained well, and when chloroformed on the 14th day appeared to be normal.

Pheasant No. 9. Did not show any symptoms of disease when chloroformed on the 14th day. No infiltration visible at the site

of inoculation. Internal organs appeared to be normal. Agglutination test positive in a dilution of 1:80. Three guinea pigs inoculated respectively with blood, liver and breast muscle remained well and when chloroformed on the 14th day appeared to be normal.

Pheasant No. 10 appeared to remain perfectly well until it was chloroformed on the 18th day. No infiltration at inoculation site. Internal organs apparently normal. Agglutination test positive in a dilution of 1:160. Three guinea pigs inoculated respectively with blood, liver and breast muscle remained well, and when chloroformed on the 16th day appeared to be normal.

The results obtained by skin inoculations of ring-necked pheasants indicate that tularemia infection is not easily established by such a procedure, if at all. Following the injection of *Bact. tularense* into the breast muscle, the organism may remain alive in that organ as long as 7 days, as shown by the result obtained on pheasant No. 7. An early invasion by *Bact. tularense* into the internal organs may occur, as indicated by its presence in the liver of pheasant No. 6, killed on the 4th day after intramuscular injection.

The pheasant appears to rid itself completely of the organism during a period following the 7th day, as it was not found on the 11th, 14th, 18th or 26th days. Specific agglutinins may occur in the blood stream by the 7th day following intramuscular injection, while it appears that no specific agglutinins are produced following cutaneous inoculation. In no case did a pheasant show any symptoms of disease following inoculation of *Bact. tularense.* It appears, however, that by intramuscular inoculation the organism will remain alive in the breast muscle, may invade internal organs and stimulate the production of specific agglutinins. We may say then that an experimental symptomless infection of tularemia may be produced in the ring-necked pheasant. The difficulties in establishing this infection, however, make it appear that the ring-necked pheasant would be immune to tularemia under conditions of natural infection.

4250

Iodine in Maryland Waters in Relation to Goiter.

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From the Laboratory of Physiological Chemistry, University of Minnesota.

A large amount of seafood insures an abundance of iodine in the diet and there is very low goiter incidence in the tidewater region.

It has been shown that sea-spray blown back on the land accounts for a sufficiency of iodine only in a narrow zone, about 3 to 6 miles wide, along the coast, so this source of iodine is not sufficient to account for the low goiter incidence in the tidewater region.* As shown by the Draft Board statistics and Public Health Service, goiter is prevalent in the mountainous region of Western Maryland. This is accounted for by the low iodine content of the drinking water of 3 western counties, as given below, compared with that of Baltimore.

Mountain Counties	*Towns*	*Iodine parts per billion*
Allegany	Luke	1.18
	Lonaconing	0.72
	Cumberland	0.06
	Barton	1.21
	Westernport	0.30
	Frostberg	0.46
Washington	Hagerstown	0.10
	Hancock	0.18
Frederick	Brunswick	0.10
	Frederick	0.40
	Thurmont	0.16
Tidewater County		
Baltimore	Baltimore	5.00

The iodine content of drinking water is an index of the iodine content of the local food supply.

We are indebted to Dr. Chas. B. Davenport and the Department of Health of Maryland for water samples. This research is done under a grant from the Joint Research Committee (Mayo Foundation) and the Therapeutic Research Committee, Council on Pharmacy and Chemistry, American Medical Association.

* The isochlor for 6 parts per million of chlorine in the drinking water (almost pure rain water) is but a few miles from the coast in New England and New York (Emmons, W. H., *U. S. Geol. Survey Bull.* 529, 1913). Assuming that sea water contains 20 parts per billion iodine and 2% chlorine, there is one part of iodine to a million of chlorine and the isochlor referred to is the isoiodine line of 0.006 parts per billion, which is too small to be of significance in the prevention of goiter. In fact, the iodine content of non-goiterous regions of the United States is at least 100 times as large as this figure and the iodine content of the drinking water of Baltimore about 1000 times this figure. Near San Francisco (Mitchell, J. P., *Stanford Univ. Pub.;* Univer. Series No. 3, 1910) the isochlor for 50 parts per million in drinking water varies from a few yards to 6 miles from the coast, although the chlorine in the rain water is increased by intense evaporation. Using a ratio in sea water of 2 parts of iodine per million of chlorine (Cameron, A. T., "Contributions to Canadian Biology: Studies from the Biological Stations of Canada," 1922) the amount of iodine blown from the sea in drinking water would then be only 0.1 part per billion or not greater than in the goitrous region of Maryland.

4251

Basal Metabolism (Oxygen) of Normal Women in Relation to Injection of Follicular Hormone.*

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Menformone and estrogen were obtained from the manufacturers. Ovarian hormone was extracted with ether from the urine of pregnant women. Ovariectomized mice were used in assaying the hormone.

In order to secure very accurate measurements, women were placed on weighed diets (no breakfast) and allowed about the same amount of exercise each day and metabolism taken 15 hours after the last meal. The O_2 and CO_2 were determined by the apparatus described in *J. Biol. Chem.*, 1928, lxxvii, 413, the nitrogen determined by micro-Kjeldahl, and, the basal metabolism calculated. By this means the Benedict-Roth-Collins apparatus was tested. The psycho-galvanic reflex, muscle tone, and blood pressure were measured as a check on the physiological state of the subjects. Half the women had basal metabolisms 15% below Aub-Dubois' standard, probably due to deficiency of iodine during childhood. In testing the effects of hormones, it was found that 1 mg. of thyroxine subcutaneously raised the basal metabolism of "normal" women 3% in 2 days and 5 mg. raised it 15% in 2 days. Injections of "menformon" and "estrogen" were made subcutaneously. On days of injection no food was taken and hence the physiological state may not have been considered absolutely constant. The basal metabolism of woman No. 1 was considered too variable for conclusions to be made and the study of her metabolism was discontinued. Women of small vital capacity had more regular breathing than women of large vital capacity.

After injection of 2000-4000 mouse units of ovarian hormone it is excreted in the urine in about 6 hours.

In the first woman the metabolism rose after injection of 300 mouse units and fell after 880 units. In the 2nd woman the metabolism fell after 1000 mouse units. The metabolism of the 3rd woman remained nearly constant all day with no injection, but began to rise 7 hours after injecting 1000 units. The metabolism of

* This research was aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

TABLE I.

Woman	No. of mos. met. was studied	Day	Hour	Cal. per sq. m. per hr.	Injection mouse units
1	1	1	1	30.0	
		2	1	—	150- 300
			5	31.7	
			5¼	35.2	
		3	1	—	440- 880
			1½	31.9	
			2	30.9	
			4¼	30.0	
2	4	1	1	35.5	
		2	0	35.8	
			1	—	500-1000
			2	34.4	
			4	34.4	
			6	34.1	
			8	33.7	
		2	1	30.5	
3	4	1	1	31.0	
			3	30.1	
			5	29.6	
			7	30.6	
			8	31.3	
			9	32.8	
		2	0	32.4	
			1	—	500-1000
			1½	32.4	
			2	30.7	
			3	32.2	
			4	31.7	
			5	32.8	
			6	32.8	
			7	34.2	
			7½	35.0	
			7¾	35.6	
		3	1	32.7	
4	2	1	1	35.0	
		2	0	35.0	
			1	—	1000-2000
			2	34.0	
			3	35.2	
			8	38.0	
			9	37.1	
		3	1	35.9	
5	2	1	1	31.8	
		2	0	32.1	
			1	—	2000-4000
			3½	32.4	
			4	33.5	
			5	32.6	
			6	33.0	
		3	1	31.6	

the 4th woman rose after injecting 2000 units. The metabolism of the 5th woman remained nearly constant after injecting 4000 mouse units.